THE MYCOLOGY OF THE MOUTH

THE

MYCOLOGY OF THE MOUTH

A TEXT-BOOK OF ORAL BACTERIA

BY

KENNETH WELDON GOADBY

D.P.H.Camb., L.R.C.P., M.R.C.S., L.D.S.Eng.

Bacteriologist and Lecturer on Bacteriology, National Dental Hospital; Sen. Dental Surgeon, Seamen's Hospitals; Hon. Lecturer on Hygiene of the Mouth, London School of Tropical Medicine; late Demonstrator of Practical Dentistry, Guy's Hospital Dental School

WITH ILLUSTRATIONS

LONGMANS, GREEN, AND CO.

39 PATERNOSTER ROW, LONDON

NEW YORK AND BOMBAY

1903

To the Memory

of

Dr. J. W. WASHBOURN, C.M.G., F.R.C.P.

PHYSICIAN TO GUY'S HOSPITAL AND THE LONDON FEVER HOSPITAL; LECTURER ON BACTERIOLOGY IN THE MEDICAL SCHOOL OF GUY'S HOSPITAL.

MY TEACHER AND FRIEND.

PREFACE.

The micro-organisms of the mouth include species belonging to higher orders than the Bacteria or Schizomycetes. I have therefore used the wider term Mycology, in preference to Bacteriology, as the title of the present work.

Bacteriology has developed to such an extent that at the present time some fifteen hundred organisms have been described and a large proportion of them are to be met with occasionally in the mouth. It is impossible to include in the scope of the present work, all the bacteria found from time to time in the oral fluids, especially as environment, food, dust and other causes determine to a great extent the species of the buccal flora; at the same time a certain number of organisms, many of them well known to bacteriologists in other situations, are so frequently found that they deserve consideration as mouth bacteria; a few bacteria and some higher forms related to the Hyphomycetes are to be found in the mouth only, I have therefore included in the following pages those bacteria frequently living in the buccal cavity although they are also found in other places, and those special organisms so far known in no other region than the mouth. An attempt is made for the first time to produce a practical text-book dealing with mouth bacteria, and although primarily intended for the use of students of dental surgery it is hoped the collection of facts related to the Mycology of the Mouth may be of assistance to those engaged in research. As the work is mainly written for students a good deal of stress is laid upon the practical details of laboratory routine, bacteriology requiring more laboratory experience perhaps than any other subject. General principles of biology, sterilization, &c., are also given at some length, for only with a thorough knowledge of general principles is it possible to attempt the study of individual organisms and the student is advised to

master general facts before proceeding to minute descriptions of the organisms themselves.

In bacteriological work a definite plan should be followed both for the benefit of the student and for the interests of the science at large; recognised methods should always be adopted in the first place, and full details of others accurately given, the plan given in the text and the suggestions on the "Study of Cultivations," will, it is hoped, prove useful guides.

The pathogenic bacteria of the mouth are given at some length and are of great importance in the relation of pathological conditions of the mouth to general disease, a relationship long since recognised by dental surgeons competent to judge, although the subject has only recently received the general attention it deserves. Mouth pathology with probable, but so far undescribed bacteriology, is only too common and it is hoped that by calling attention to the gaps in our knowledge others may be induced to investigate these undetermined problems.

Several of the organisms given in the latter portion of the book may possibly be synonymous, but as so far I have had no opportunity of carefully testing all, the descriptions originally given are adhered to. The question of immunity is touched upon and a general statement of fact given, but it is impossible within the scope of a text-book of this description to discuss the matter in full; my intention is rather to give the main points without going deeply into the subject.

Fermentation and Dental Caries are so closely related that a good deal of space is devoted to their consideration; a general *resumé* of dental caries from the point of fermentation physiology is naturally associated with the description of the bacteria involved in what is a special form of metabiotic putrefaction.

In dealing with the special mouth bacteria references are given to the original papers to which the student has the opportunity to refer if he so wishes.

In the appendix are given some practical hints on the choice and use of the microscope, and the scheme for a system of describing cultivations of bacteria recently suggested by Chester in his "Determinative Bacteriology," definite but general terms taking the place of lengthy descriptions, the latter often proving inaccurate from the variation of individual organisms. The system has much to commend it.

In writing this book the various standard text-books have been freely consulted, among them may be mentioned: "Sternberg's Bacteriology," "Macfarland's Pathogenic Bacteria," "Muir and Ritchie's Text-book of Bacteriology," "Miller's Micro-organisms of the Human Mouth," and the works of Lefar, Hueppe, Du Barry, Pflugge, Migula, Lehman and Neumann, and others.

In conclusion, I acknowledge with many thanks the loan of a number of illustrations, which are duly acknowledged.

My thanks are also warmly accorded to my friend, Dr. J. W. Eyre, for many valuable suggestions and for the performance of many inoculation experiments at various times.

Mr. Cyril Hill has also kindly assisted in photographing apparatus and in the matter of proofs.

KENNETH W. GOADBY.

Bacteriological Laboratory,
National Dental Hospital, London.
November, 1902.

CONTENTS.

LIST OF ILLUSTRATIONS.

THE MYCOLOGY OF THE MOUTH.

CHAPTER I.

BACTERIA are minute unicellular organisms forming the lowest group of the Cryptogams, or flowerless plants, and are the intermediate link between the animal and vegetable kingdom of living things, related on the one hand to the Mycetozoa, or animal fungi, on the other to the Algæ. They are divisible into two groups, a higher and a lower. The lower, known as Schizomycetes, or fission-fungi, are most numerous, comprising the greater number of the organisms with which pathological mycology deals. They are all microscopic in size, and are rarely more than $\frac{1}{25000}$ in. in one direction. To facilitate general description mycologists have adopted a standard of measurement designated *micron* = $\frac{1}{1000}$ part of millimetre, or $\frac{1}{25000}$ part of inch, which is written μ, the dimensions of an organism being expressed as multiples or fractions, *e.g.*, "2·5 μ long, 0·75 μ wide." The lower group of bacteria consists of the relatively monomorphous varieties, which are classified according to their shape: (*a*) small globular bodies, occurring singly or associated with others, designated cocci; (*b*) rod-shaped forms known as bacilli; (*c*) spiral or corkscrew forms, and the curved fragments of the same, called spirilla. The term *bacterium* is properly applied to micro-organisms of the schizomycetal group generally, and is used as such in the present work. The higher bacteria are relatively pleomorphous, and may exhibit real or pseudo-branching as in *Cladothrix dichotoma;* their method of reproduction as well as their form is allied to the moulds, whilst some of the stages in their life cycle are

indistinguishable morphologically from the lower group of fission-fungi.

The bacteria as a group are most active chemical agents, splitting up effete animal and vegetable matter into bodies assimilable by plants, fixing free nitrogen as on the roots of Leguminosæ, and assisting in the disintegration of the hardest rocks. A limited number produce disease in both animals and plants, and finally van Tieghem claims to have demonstrated the presence of the bodies of bacteria in coal, where their activity was concerned in the rotting of the old coal forests.

Classification.—Bacteria belong to the vegetable kingdom, and are placed under the sub-group of Thallophytes, one of the divisions of the Cryptogams, thus:—

I. *Thallophytes.* — Simple plants, without leaves, stems, roots, or vascular bundles.	(1) Fungi ..	Devoid of chlorophyl.
	(2) Algæ ..	Containing chlorophyl.
II. *Bryophyta.*—Mosses, with leaves and stems, devoid of true roots and vascular bundles.	(3) Hepatinæ ..	Liverworts.
	(4) Musci ..	Feather mosses.
III. *Pteridophyta.*—Vascular cryptogams, with leaves, stems, true roots, and vascular bundles.	(5) Equisetinæ	Horse-tails.
	(6) Lycopodinæ	Lycopodium.
	(7) Filicinæ ..	Ferns.

The fungi may be divided into two main groups according to their mode of growth.

Fungi	Schizomycetes ..	Fission-fungi.
	Eumycetes	Higher fungi, generally branching.

The above scheme gives the general position of the bacteria, but it is extremely difficult to properly classify all the organisms generally included in the term "bacteria"; more particularly is this the case with those species—of which Actinomyces may be taken as an example—which are related to the true Schizomycetes on the one hand and to the Eumycetes on the other.

Most of the forms with which bacteriology has to do belong however to the Schizomycetes, and it is necessary for convenience of description to adopt a classification.

The grouping generally adopted is Baumgarten's, a modification of that first suggested by Cohn, based upon morphological form, and although not entirely scientific is at present the most convenient.

Baumgarten's classification is as follows :—

I.—Cocci. II.—Bacilli. III.—Spirilla.	Species relatively monomorphous.
I.—Spirulina (Hueppe). II.—Leptotricheæ (Zoph). III.—Cladotricheæ (Cohn). IV.—Streptothrix.	Species relatively pleomorphous.

Various other methods of classification have been suggested by different observers; thus Du Bary and Hueppe adopt a classification based on spore formation: (1) those bacteria forming endogenous spores; (2) those forming exogenous or arthrospores. Our knowledge of sporulation is as yet too imperfect to adopt such as a method of classification.

Another source of confusion is the different meaning which authors attach to their terminology; for instance, bacterium is used as a general term including bacteria generally; Hueppe uses it in the limited sense of those organisms which do not produce endogenous spores, the term bacilli being applied to the spore-forming species. Migula calls motile organisms bacilli, non-motile ones bacteria. I have adopted the commonly accepted meaning of bacillus as a rod-shaped organism, and the term bacterium or bacteria as a general one.

Considerable confusion also exists regarding the loosely applied term leptothrix, some meaning thereby a thread or special morphological form which is common to a considerable number of species. Zoph, who first used the term, applied it to a distinct species of the higher bacteria, and it is in this sense it is used in the following pages.

Chester ("Determinative Bacteriology") suggests the following classification:—

COCCACEÆ.

I.—Cells unbranched, or showing only false branching as in Cladothrix.

(*a*) Cells globular, becoming slightly elongated before division, which takes place in one, two, or three dimensions.

BACTERIACEÆ.

(*b*) Cells short or long, cylindrical, straight, curved or spiral, without sheath; motile or non-motile; endospores present or absent.

CHLAMYDOBACTERIACÆ.

(*c*) Cells surrounded by a sheath and arranged in elongated filaments.

BEGGIATOACEÆ.

(*d*) Cells not surrounded by a sheath, arranged in filaments and motile by means of an undulating membrane.

MYCOBACTERIACEÆ.

II. Cells short or long, cylindrical or filaments, clavate, cuneate, or irregular in form. Without endospores, but with formation of gonidia-like bodies by segmentation of the cells. Without flagella. Division at right angles to the

rod or filament. Not possessed of sheath, but having true dichotomous branching.

For grouping of species see Chester ("Determinative Bacteriology," p. 54).

Lehmann and Neumann suggest the following arrangement:—

(1) *Coccaceæ.*
(2) *Bacteriaceæ.*
(3) *Spirillaceæ.*
(4) *Corynebacterium*, Diphtheria bacillus.
(5) *Mycobacterium*, acid fast organisms (tubercle bacillus).
(6) *Actinomyces.*

Cladothrix, Crenothrix, and Leptothrix are classed as "Higher fission-fungi."

In both these classifications the genus Bacteriaceæ are divided into two classes:—

(*a*) Bacterium—not forming endospores.
(*b*) Bacilli—forming endospores.

For further information see Lehmann and Neumann (p. 119 *et seq.*), where proper principles for nomenclature of bacteria are laid down.

The Lower Bacteria.—(*a*) Cocci—round, oval, or elliptical cells ranging from 0·5 to 2 μ in diameter. When not spherical the greatest diameter is not greater than twice the lesser. They are not possessed of motility but often exhibit considerable Brownian movement (p. 11) with the exception of a few species (*e.g.*, Micrococcus agilis of Cohn, Malta fever coccus). Reproduction is by binary fission, and spore formation (endogenous) is unknown. In some cocci, particularly the streptococci, large swollen elements with increased refractive power are often to be seen; these have been described by Du Bary as a mode of sporulation under the term arthrospores (fig. 1, *d*.)

The cocci are arranged into groups according to their mode of reproduction.

(1) Streptococci.—Division (binary fission) occurring regularly in one plane only, the individual elements remain attached by their capsules in the form of chains; in some species the chains may attain great length and be composed of a large number of individual cocci.

(2) Staphylococci.—Division occurring irregularly in one plane only, the cocci remaining attached by their capsules in irregular clumps and masses, compared to bunches of grapes.

The number of species in these two groups is very large. The formation of arthrospores is well marked (Du Bary).

(3) Diplococci.—Division in one plane regularly, the cocci

remaining associated in pairs; both of the foregoing groups exhibit this form, the term being applied to a given species when it occurs most commonly in the diplococcal form (Diplococcus pneumoniæ).

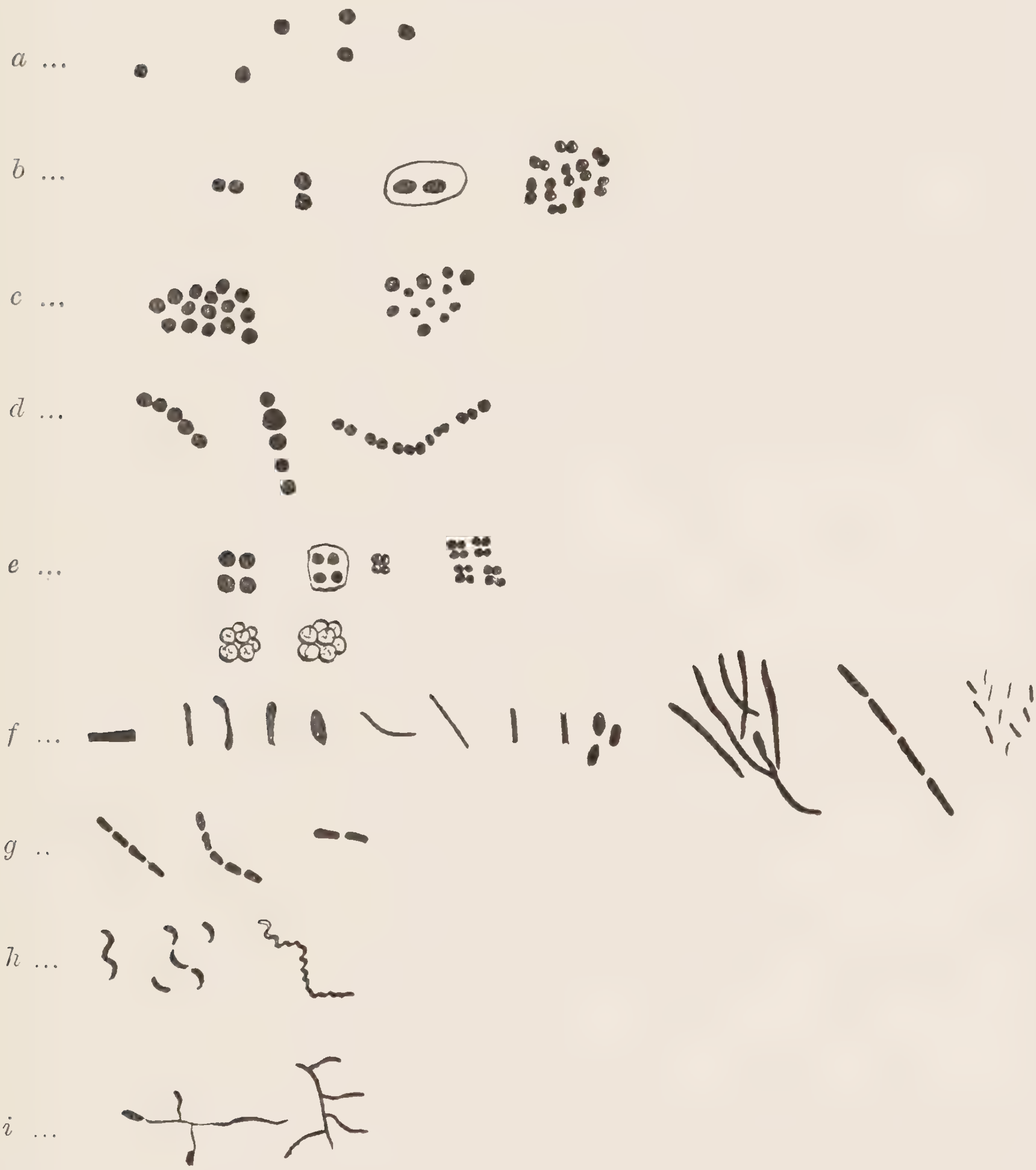

FIG. 1.—MORPHOLOGICAL FORMS OF BACTERIA.

a, Cocci; *b*, diplococci, diplococcus with capsule; *c*, staphylococci; *d*, streptococci; *e*, tetracoccus and sarcinæ; *f*, various types of bacilli; *g*, streptobacilli; *h*, spirillum, comma forms and spirochæte; *i*, streptothrix branched threads.

(4) MERISMOPEDIA.—Division in two directions in the same plane, the cocci remaining attached in groups of four, or "tetrads," as Micrococcus tetragenous.

(5) Sarcinæ.—Division in three planes, one at right angles to the other two, the cocci remaining attached in cubical packets of eight, which are often associated in masses. These cocci are generally of larger size than the other groups.

The term *micrococcus* is applied to all the above species, and is also used to designate cocci occurring separately, as mono-cocci; such mono-coccal form is common among the staphylococci.

(*b*) Bacilli.—Rod-shaped organisms, the greatest diameter being more than twice the lesser; the cells may be long or short, but, as a rule, are not wider than 1·0 μ. Division generally takes place at right angles to the long axis. The cells of some species have flagella and are motile. Two bacilli may remain united by their capsule, forming a *diplobacillus*, or the individual elements remaining united in chains a *streptobacillus* is formed. These two morphological forms are not so constant as the corresponding cocci, and are of little use in classification. Bacilli frequently grow out into long threads, particularly in fluids, to which form the term "leptothrix" is often wrongly applied. Endogenous spore formation occurs in many species. The spores may be central or terminal in position; round, oval or fusiform in shape. In the latter case the organism containing the spore becomes swollen and spindle-shaped and is termed a *Clostridium*.

(*c*) Spirilla.—Curved or comma-shaped rods and spiral filaments. Reproduction by binary fission or simple division. Generally motile, movement in direction of long axis and rotation upon same axis. In the long spiral threads, also termed *spirochæte*, the motility does not always appear to be due to the presence of flagella, the organisms possessing contractility. The comma-shaped cells generally possess flagella, which may be single or multiple and situated at one or both poles.

The term "vibrio" is often applied as a generic term to this group; some authors however use "vibrio" in a special sense, thus Hueppe calls forms without endospores "spirochæte," "vibrio" those having endospores. Migula applies the term *vibrio* to those organisms with only one or two polar flagella, *spirilla* those with bunches of flagella, and *spirochæte* those without flagella. Flügge employs "vibrio" to denote forms with but slightly marked undulations.

The Higher Bacteria.—These organisms show a distinct advance on the lower group, but at present our knowledge of them is fragmentary and includes only isolated species. Some of the species show differentiation of the two extremities; one end

may be specialised for attachment, the other for reproduction—a method approaching the sporulation of the Moulds, to which the High Bacteria are somewhat closely related. The filaments of these bacteria are generally segmented, but special methods are required to bring out the divisions. There is often a capsule common to the whole thread.

Spirulina (Hueppe).—The cells are sometimes rod-shaped, sometimes spiral, and in some media may grow out into long spiral, undulatory or straight filaments. The threads break up into cocci-like reproductive bodies—"arthrospores." Under this head the Proteus group was first described; they are now generally placed with the bacilli.

Leptothrix (Zoph).—Rod-shaped, spherical and filamentous forms, the last showing a difference between base and apex. Filaments straight or spiral. Spore formation unknown.

This definition of leptothrix is the one I have adopted in the following pages, bacilli forming filaments or threads are not included in it. (See later.)

Cladothrix.—Spherical, rod-shaped, and filamentous forms, the latter show pseudo branching. Reproduction by arthrospores.

Streptothrix.—Felted mycelium-like filaments, showing true dichotomous branching. Club-shaped thickenings appear at the ends of some of the threads. Various forms are produced by breaking up of threads simulating cocci, bacilli and spirilla; from these new individuals may be formed.

Bacteria are also classified according to the environment necessary to their development, as Saprophytes and Parasites.

The *saprophytes* are those whose existence is possible apart from a living host, and which obtain the nutriment necessary for their growth from dead organic matter, or from simple organic salts and water.

The strict or *obligatory parasites* are unable to exist apart from a living host, in whose tissues they multiply, often producing profound pathological changes; some few species may exist in the tissues of an animal without any harm arising.

Such bacteria as are capable of leading a saprophytic existence, but when gaining access to the tissues of the body will develop there, are termed *facultative parasites.*

Of the obligatory parasites the leprosy bacillus affords a good example; all attempts at its culture have failed, and it is unknown

apart from the disease with which its name is associated. The cholera spirillum, typhoid bacillus, and most pathogenic bacteria are examples of the facultative parasite, which, besides the production of disease by development in living animal tissues, is enabled to exist outside the body as a saprophyte.

There are many transitional forms between the obligatory parasite and the saprophytic bacteria; many of these have only been obtained in pure culture within recent years, as, for instance, the influenza bacillus and the gonococcus. An organism living a parasitic existence as a general rule does not grow in artificial media as well as one which has for some time led a purely saprophytic existence. For this reason many bacteria require some little time before they develop their "laboratory habit." It seems not improbable that the various pathogenic bacteria which are to-day associated with disease were at one time simple saprophytes, and that some of the simple saprophytes, as we know them to-day, may yet attain pathogenic powers.

Not to admit such a development of pathogenic power entails the obscession that pathogenic bacteria were created by design to destroy human life, and, moreover, such a refusal places us at variance with the Monistic conception of the universe, and the orderly operation of the laws of evolution with which all observed phenomena accord.

Morphology has been already referred to as the general basis of classification. Morphological form, however, is extremely variable, so that only the predominating or average form in any given species is taken as representing that species. Thus, for instance, the Pneumococcus or Diplococcus pneumoniæ occurs generally in the form of diplococci, but also constantly presents a monococcal and streptococcal form. The streptococcus of the mouth occurs in that cavity as a diplococcus almost without exception, whilst in the majority of culture media the streptococcal form predominates. Under certain circumstances the individual cocci may become so much elongated that a form allied to a strepto-bacillus is produced. It is for this reason, as well as for the fact that three chief morphological forms are common to a great number of families, that mycologists have adopted the methods of cultivation in the determination of species.

Chemistry.—The determination of the chemistry of the bacterial cell was first undertaken by Nencki and Schäffer, who found that

the bodies of bacteria were very rich in a nitrogenous substance to which the term micro-protein was applied; with nitric acid this body does not give the xanthoproteic reaction. The percentage of nitrogen is generally about 14·75.

The bacterial bodies also contain some 3·5 per cent. of fat; Bullock has recently extracted a considerable quantity of fat from tubercle bacilli. Bacteria elaborate within their own protoplasm various ferments or enzymes and poisons or toxines; some of these are retained within the bacterial cell and may be obtained from the washed bodies of bacteria by appropriate methods. Thus Buchner obtained an active body, which fermented sugar with the production of alcohol, by expressing the contents of washed and dried yeast. The fermentation produced in no way differed from that brought about by living yeast, the fluid being demonstrably free from living organisms. Quite recently Macfadyen[1] and Rowland prepared a principle from the washed bodies of typhoid bacilli by triturating them with sterile sand in a special apparatus. The glycerine extract obtained when injected into rabbits induced an agglutinative power in the serum similar to that developed during an attack of typhoid fever in the human subject. Some bacteria give a blue colouration with acidulated iodine, due to the presence of granulose; a number of these are met with in the mouth at various times. The butyric acid-bacilli give this blue reaction with iodine, and are grouped generically as *Granulobacteria* (Lefar).

Gram's method of staining (p. 46) also depends on some chemical difference in the composition of the given organism, some retaining the stain, others are quickly decolourised with the alcohol used.

Structure.—The bacterial cell consists of a thin cell wall enclosing clear and, as far as is at present known, structureless contents.

The cell wall, allied but not identical with cellulose, is said to be of a radiate, honeycombed appearance, and that in this layer is situated the colouring matter of the chromogenic organisms, whilst the sulphur granules appearing in the Beggiatoa are contained in the internal plasma.

The plasm also contains at times highly refractile granules which are not spores; these granules are said to be homologous with the chromatin granules of higher plants from the avidity with

---

[1] *Cent. für Bak.*, xxiv., 1902.

which they take up colouring matter—such granules are to be seen in the diphtheria bacillus.

By a delicate process of extraction and subsequent staining with hæmatoxylin the central plasm of the organism has been shown by Bütschli to be finely reticulated, and that the plasm appears differentiated into a central portion and a parietal layer besides the cell wall; the central mass is therefore a large nucleus, the parietal layer corresponding to the cytoplasm of higher plants.

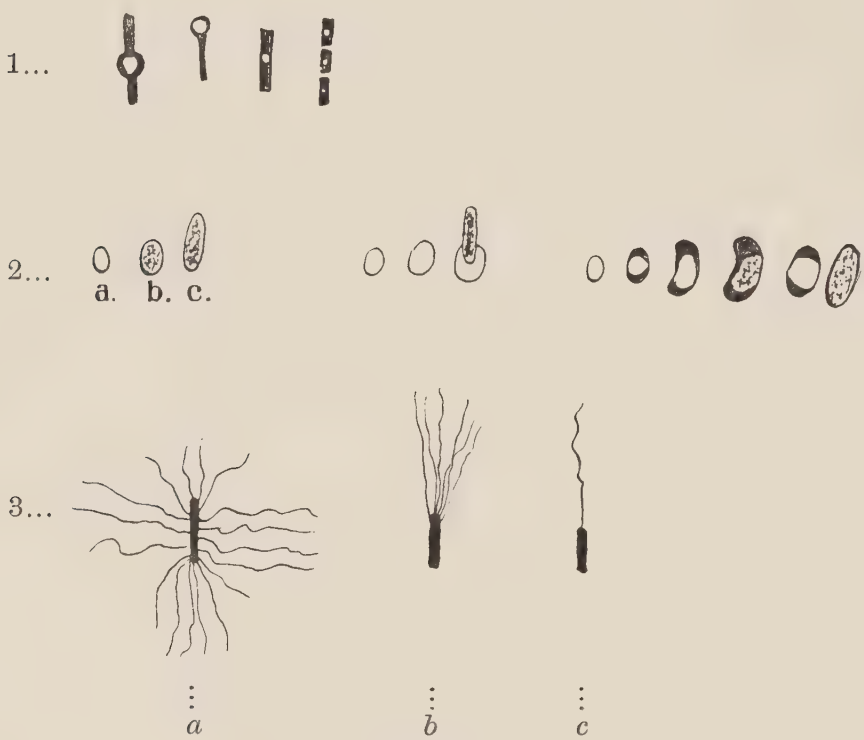

Fig. 2.

1, Types of spore-forming bacilli; 2, three types of spore germination; 3, flagellæ: (*a*) peritrichic, (*b*) lophotrichic, (*c*) monotrichic.

Most bacteria are surrounded with a gelatinous, homogeneous covering termed *capsule;* in some species—as the pneumococcus of Fränkel and the pneumobacilli of Friedländer—it may be easily demonstrated. It is not always present in cultivations, and is seen best in preparations made from the blood of an animal which has succumbed to infection with the organism. The capsule is also seen in specimens obtained from pneumonic lung and "prune juice" sputum.

Many organisms possess the power of independent movement and are described as motile. Upon such organisms extremely

slender whip-like filaments may be observed by special methods of staining, known as flagella, and may be single and situated at one end, or the organism may be thickly studded with them; they are often many times the length of the organism. Owing to their minute size it is difficult to say to which part of the bacterial cell they are primarily attached. With the exception of a few doubtful species motility is confined to the bacilli and spirilla. By examination of hanging drop preparations the motion can be easily observed, and the organisms seen darting about in all directions. The absence of motility is not always synonymous with absence of flagella, many physical conditions causing "torpidity."

Bacteria possessing flagella are classed as (*a*) *Peritrichic*, multiple flagella surrounding the bacillus; (*b*) *Lophotrichic*, tufts of polar flagella; (*c*) *Monotrichic*, single polar flagella. (Fig. 2.)

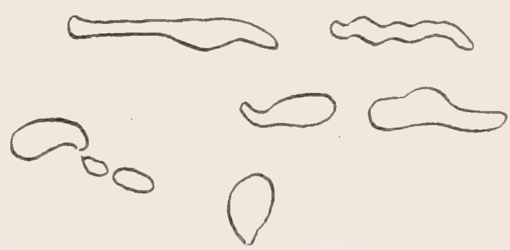

Fig. 3.—Involution Forms of Bacteria.

Another form of movement which must not be confounded with the above is what is known as "Brownian movement," consisting of a dancing, oscillating swing of the organisms. It is a purely physical condition occurring with solutions of inorganic matter, exactly as with non-motile organisms, and is probably related to surface tension. In determining motility, observe the position of three organisms situated at the angles of an imaginary triangle and watch for a change of relative position, carefully excluding mechanical shock.

Spore Formation.—Various organisms of the class bacilli produce within their plasm highly refractile bodies which are capable of withstanding higher temperatures and stronger disinfectant solutions than the vegetative form; these bodies are spores.

The spores take up staining reagents with great difficulty, special treatment being necessary (see chapter on microscopical methods). Spore formation consists of a condensation of the cell

plasm, the spore thus formed being surrounded with a tough membrane consisting of two layers. At the same time the remainder of the organism undergoes degenerative changes, eventually setting free the mature spore. Certain other clear spaces exist from time to time in the bacterial plasm, filled with lustrous drops of a fatty nature rendering observation alone useless in the determination of sporulation (page 47).

The number of spores formed by a bacillus is rarely more than one, and is sometimes situated at one end, when the "drum stick" form is produced (Tetanus bacillus, fig. 2, 1).

In some species (B. alvei), when the spore is centrally situated the cell plasm alters in form and becomes massed around the spore, forming a clostridium or spindle-shaped mass.

The form and size of the spores differ greatly in various species but is constant for the same species. B. subtilis has ellipsoidal spores 1·2 μ length by about 0·6 μ breadth.

These spores formed in the interior of bacteria, or endospores as they are termed, are highly resistant to the action of heat, disinfectants, or light, and a proper knowledge of them is of supreme importance in all bacteriological and hygienic work.

Gruber and Brefield found that the spores of B. subtilis required three hours' boiling at 100° C. to kill them, whereas the rod-shaped forms were easily destroyed by heating for twenty minutes at the temperature of boiling water. When the spore is exposed to favourable conditions it germinates, and in a short time (three to four hours for B. subtilis, Prazmouski) produces the parent form. The process is of three types:—

(1) *The spore gradually elongates*, the outer membrane disappearing, eventually reaching the adult form which divides by binary fission, in the resulting cells spore formation again taking place.

(2) *The spore membrane is ruptured* at the point of least resistance, and gradually grows out from the empty spore capsule, which dissolves in the surrounding fluid. The capsule, especially in motile forms, may be seen attached to the free-swimming rod.

(3) *The spore membrane ruptures at the equator of the spore*, the developing rod gradually forcing its way out by one pole; a portion of the spore may remain attached.

Plasmolysis, or shrinking of the cell plasm with resulting spaces between the plasm and cell wall, may be produced by the action of

certain reagents, among them being the ordinary physiological salt solution (0·75 gm. NaCl in 100 gms. of water). Various staining reagents bring about this phenomenon. The plasmolytic effect may be produced and removed without any apparent injury to the living cell.

CHAPTER II.

The Biology of Bacteria.

Bacteria are greatly influenced by their surroundings, and on the other hand often profoundly modify the substratum in which they are growing, be it living tissue or nutrient solution, whilst the very products resulting from their activity are among the chief inhibitory influences restraining their indefinite development.

Cohn estimated that a single bacillus 2 μ long and 1 μ broad, weighing 0·000000001571 mgrm., and which reproduced itself by binary fission once in half an hour, will in two days' time have a progeny of 281 billions, occupying a volume of half a litre, while in another three days the mass would be sufficient to fill the beds of all the oceans of the globe, the number of the progeny being represented by 37 places of figures! That such enormous development does not take place is due partly to the antagonism displayed by one species towards another, partly by the insufficiency of nutriment obtainable, but chiefly to the products of the organisms' own activity. The more important conditions related to the development of bacteria are—light, temperature, gaseous environment, moisture, food supply. The first four factors are related more particularly to the development of the bacteria, whilst the question of food supply is largely complicated by the various chemical changes induced by bacterial action.

Effect of Light.—The antagonism of light to disease was a fact established by empirical observation long before bacteria were known to exist, and the old Italian proverb, "Where the sun does not enter, the doctor does," is illustrative of this popular knowledge gained by observation. Since the discovery of bacteria many experiments have proved the scientific basis of the empirical deduction; the majority of bacteria, and certainly all the known pathogenic forms, are particularly sensitive to the action of light.

Direct sunlight is the most powerful agent; exposure to the

direct rays of the sun will kill tubercle bacilli in half an hour, anthrax spores in an hour. Diffused daylight has a similar, but less energetic effect, the time of exposure being about three times as long. The light from an electric arc has a similar effect to diffused daylight; recently advantage has been taken of the fact in the treatment of lupus.[1] Tuberculous sputum and typhoid dejecta may thus be deprived of their respective organisms under natural conditions. Even when the organisms are not entirely destroyed by the process of insolation their pathogenic powers are greatly attenuated, the subsequent cultivations developing less luxuriantly than before the experiment, and the power of producing poisonous products greatly diminished.

Among the bacteria producing pigment, light has marked effect in altering the power of chromogenesis. Thus if a cultivation of the B. rouge d'Kiel—an organism producing a fine red pigment—be exposed to sunlight for a time insufficient to entirely destroy the organisms, subsequent cultures may be obtained which have lost the power of colour-formation and remain colourless indefinitely. A few non-pathogenic bacteria, such as *B. violaceous*, which forms a purple pigment, apparently thrive best in the light. The red chromogens are generally more resistant to the action of light than other colour-forming species.

Downes and others have attributed the destructive action of sunlight to a disengagement of nascent oxygen which attacks the bacterial plasma, such a process depending largely on the composition of the medium containing the bacteria during exposure. The blue and violet portion of the spectrum, *i.e.*, the most chemically active rays, were found most energetic in action, the red and yellow rays the least. The destruction was apparently independent of the temperature, and took place when the heat rays were excluded. The more translucent the medium the greater the action.

Buchner found in a series of experiments conducted in the clear water of Lake Starnberg, that the bactericidal effect of light was apparent at the depth of two metres below the surface. Sunlight must therefore exercise a powerful influence in cleansing water-courses polluted with excremental matter; the marked diminution in the number of bacteria present, say, a mile below a sewage

---

[1] *British Medical Journal*, January, 1902.

outfall, undoubtedly depends upon such action, besides sedimentation and other processes.

Moisture.—Water is necessary for bacterial development as for other forms of life; the optimum percentage of water is about 80 per cent. Some bacteria will withstand desiccation for long periods, others rapidly succumb; the spore-bearing organisms resist drying to a much greater degree than the non-sporulating varieties, the arthrosporous forms holding an intermediate position (Hueppe). Anthrax spores will survive drying in dust for two years or more. A cultivation of B. typhi abdominalis kept in my laboratory for nine months, and which had become so dry that the medium could be broken with ease, gave subcultures in twenty-four hours.

B. diphtheria will resist drying for a week or two. Tubercle bacilli remain alive for long periods in rooms occupied by tubercular persons, especially in dark situations, as behind pictures, &c. On the other hand the cholera spirillum is destroyed by three to four hours' drying.

Most Schizomycetes exhibit their maximum development upon fluid media, bacilli often growing out into long chains of filaments: "dry rot" and "mould" are always associated with dampness.

Relation to Gaseous Environment.—Bacteria, in common with other living things, require oxygen for their existence, but not in all cases is it necessary that the gas should be present in the free state, as certain organisms can obtain the necessary oxygen from chemical compounds in which it is present in a loosely combined form.

Such organisms are termed *anäerobic*, that is, they will live even though free air is excluded; others however require oxygen in a free state, and are termed *äerobic*. Intermediate between these two extremes come those organisms which, although they develop best in the presence of air, are yet capable of existence when air is excluded; such are termed *facultative-anäerobic*, and comprise the largest number of the known Schizomycetes.

Anäerobiosis is probably an ancestral trait going back to the first appearance of life upon this planet, when the atmosphere contained but little oxygen in a free state. It is possible experimentally to change the character of an organism that, though at first it is äerobic, subcultures will ultimately become anäerobic in habit, and *vice-versâ*. Such experiments have been performed by Hueppe with the cholera spirillum.

Hydrogen and nitrogen are indifferent gases for anäerobes, while sulphuretted hydrogen and carbon dioxide are poisons. On the other hand the majority of mouth bacteria are able to develop in an atmosphere of carbon dioxide—in fact certain species are favoured by its presence.

The Beggiatoa group, as observed by Winogradsky, are able to exist in sulphuretted hydrogen, and change that gas into its component parts, storing up the sulphur in solid particles.

The changes induced in the substratum by anäerobic bacteria differ from the changes taking place in the presence of free oxygen. The maintenance of life without free oxygen depends solely upon the availability of compounds from which oxygen may be split off.

"The amount of chemical change therefore is relatively much less intense than in äerobic conditions; thus if 1,000 grammes of sugar be completely oxidised to CO_2 and water in the presence of free oxygen, 3,939 calories or heat units are produced; if however it is split into butyric acid, hydrogen and CO_2, only 414 calories are evolved. It follows therefore that anäerobic bacteria must superficially disintegrate a far larger quantity of material to obtain this necessary oxygen than äerobic organisms—a circumstance that has considerable significance in the large production of toxines by organisms growing in the living body. Again, during anäerobic fermentation the secondary products are not oxidised to other and simpler compounds, and they therefore accumulate, a good example being afforded by the so-called "bottom fermentation."[1]

It has also been shown by Fajans[2] that cholera cultures retain their virulence much longer under anäerobic than under äerobic conditions; and Braatz[3] has called attention to the fact that bacteria in suppuration foci are living without atmospheric oxygen.

A great pressure of carbon dioxide is said to deprive B. anthracis of the power of sporulation.

It is probable that facultative anäerobic organisms are largely concerned in dental caries after the granular layer has been passed, and the rapid progress and undermined character of the cavities generally formed is due to the phenomena connected with anäerobiotic growth; gelatin, the end-product of the tooth cartilage or collogen, is a substance from which anäerobes are able to obtain their oxygen. A relatively large amount is therefore attacked, and in these anäerobic cavities much more of the matrix has

[1] Hueppe, "Princ. of Bact.," p. 54.
[2] *Arch. f. Hyg.*, xx.
[3] *Deutsche med. Wochensch.*, 1890, No. 46.

disappeared than is the case in those cavities to which oxygen has free access.

Temperature.—Bacteria are the most widely distributed of all living things when we consider them from the point of view of temperature.

Foster[1] and Fischer[2] have demonstrated that a number of bacteria, amongst them the species producing phosphorescence, thrive and multiply at 0° C. *Per contra* Miguel has described an organism that flourishes and forms spores at 70° C. (158° F.), a temperature at which ordinary albumen is coagulated! These two extremes however only give the limits of actual growth, the limits of passive resistance being much wider. For instance, Pictet[3] found spores to resist exposure to the temperature of frozen oxygen (—213° C.) for a short time, and that they easily withstand a temperature of — 120° C. for twenty hours, rapidly developing when thawed. Rapid freezing and thawing was found to be more injurious than a long exposure to a low degree of cold.

The ordinary bacterial plasm of most organisms enters into heat rigor at 42° to 45° C., and a prolonged temperature of 55° C. will destroy almost all bacterial bodies; but the fact does not apply to the spores, those of B. subtilis requiring three hours' continuous boiling in water or steam to destroy them. The point at which death occurs is termed "thermal death point," and varies considerably for various species. The property of resistance to temperature as high as boiling was one of the experiments by which bio-genesis was sought to be proved among others by van Helmont, who devised therefrom a process of producing artificial mice!

The spores present in the boiled fluid develop into adult forms as soon as the temperature has fallen sufficiently. From this, and the fact that the bacterial bodies themselves were easily destroyed by boiling, Tyndell devised what is known as *intermittent sterilization.* The medium, which would be spoiled by a high temperature, is boiled for twenty minutes on three successive days. In the interval between the operations the spores germinate to adult forms which are killed at the next boiling.

Saprophytic bacteria of soil and water grow best at about 20° C.,

---

[1] *Centralbl. f. Bakt.*, ii., 1887; xii., 1892.

[2] *Ibid.*, iv., 1888.

[3] *Arch. des Sci. Phys. et Nat.*, xxx., 1893, p. 293.

growth ceasing at about 5° C. The pathogenic organisms have their optimum temperature at the body heat of about 37° C., although each of them generally exhibits a preference of some definite degree of heat which is termed "optimum temperature."

The relative resistance of different bacteria is often made practical use of to isolate the more hardy species. It is also used in the determination of the presence or absence of endospores. The cultivation or material to be tested is maintained at a temperature of 80° C. for half an hour. At the end of the time the tube is replaced in the incubator for twenty-four hours. If spores are present they resist the action of the heat and develop rapidly when incubated; when no spores are present no development occurs.

Heat is applied to "attenuate" pathogenic cultivations for inoculation purposes. Pasteur found that by incubating the anthrax bacillus at a temperature of 40° to 42° C. no spores are formed, and the pathogenic power of the bacilli is greatly reduced. Even spores themselves if maintained for considerable periods at 80° lose their pathogenic power. The digestive and bacterial enzymes are mostly destroyed by temperatures above 70°, being more resistant than the vegetative forms but less so than the endospores.

The foregoing facts have a very practical bearing upon sterilization and will be again referred to under that heading: and in passing it may perhaps be as well to point out that freezing or cold-storage does not destroy the bacteria, but merely restrains their activity for the time being.

Reaction of Medium.—Most bacteria grow best when the reaction of the substratum is neutral or faintly alkaline, the majority of the putrefactive bacteria and most of the pathogenic bacteria are favoured by an alkaline reaction; some organisms, however, are able to grow on an acid medium, while a few are directly favoured by the presence of acid, as for instance the acetic acid bacilli, which ferment acetic acid to CO_2 and water. B. butyricus is another of these acid-loving organisms. The mouth bacteria all prefer a somewhat alkaline medium, most of them refusing to develop in the presence of acid; a few—particularly the mouth streptococcus—will grow in acid media. This ability of bacteria to develop in an acid medium must not be confounded with the production of an acid reaction by the vital activity of the organism.

The reaction of the culture medium in which bacteria are

cultivated, has considerable bearing on their development, different species showing a somewhat marked preference for certain percentages of alkalinity—in fact, different races even of a given species of organism will show differences when grown on media containing slight differences in reaction, so much so that it is often possible to pick out a given race by these means. It follows, therefore, that in all practical bacteriological work definite and careful methods of standardisation should be adopted (see chap. 4).

Food Supply.—The *rôle* of bacteria in nature is the breaking up of the complex chemical compounds of the bodies of plants and animals with the release of the chemical constituents so that they may be again recombined and utilised in building up fresh living things. A small section of the vast number of existing bacterial species have become so modified in their mode of life that they are able only to exist in the bodies of animals or plants, and developing in such situations initiate pathological changes with various symptoms peculiar to special diseases. It follows, therefore, that the food material required by one species is not always adapted to the development of another; in some cases—such for instance as the diplococcus of pneumonia, or the gonococcus—the organism can only be grown at first upon a medium smeared with fresh blood; but even these refractory organisms in time adapt themselves to their new environment, and may be cultivated upon the ordinary laboratory media. The greatest number of bacteria, however, are remarkably adaptable, and may be cultivated upon what are termed "artificial media."

An attempt is always made to reproduce, as far as possible, the natural food condition enjoyed by bacteria; but to do so exactly is generally impossible owing to the complex nature of the natural food stuffs, and moreover to the frequent presence of organisms other than the special one it is sought to isolate. In a certain number of cases, however, the artificial cultivation may present better opportunities for the growth of a given organism than is possible in its natural habitat, many organisms excreting bodies harmful to other species and also to their own development. Two organisms therefore which, when growing together, exhibit mutual antagonism, may individually grow more easily when separated in pure culture in artificial media.

Bacteria do not always antagonise one another, and many cases are known where the presence of one species of bacteria actually

assists the development of another. Thus in the well-known fermentation that takes place when the juice of grapes is expressed furnishes an excellent example. The crude wine-must when it comes first from the press contains a large and varied flora, amongst which are yeasts. These yeast forms finding the surroundings especially fitted for their development ferment the sugar present to alcohol and CO_2 until the alcohol reaches a certain percentage when they are unable to develop further. Another series of organisms now comes into play, contained like the yeasts in the original wine-must. These organisms attack the alcohol and change it to acetic acid, and as the alcohol becomes used up, cease their activity and give place to a third series, which having a special taste for acid solutions were unable to develop before their particular food was obtainable. As a result of their growth the acetic acid is fermented to CO_2 and water, and the reaction of the medium becomes again neutral or faintly alkaline. The way is thus prepared for the putrefactive organisms which have gained access from the air, or from the original grape skins; these bacteria change the remaining proteid matters into CO_2, water, nitrogen and various evil-smelling gases that generally accompany putrefaction. Such a cycle is the common phenomenon in most spontaneous decompositions. The alcohol stage may be and often is omitted, direct change of carbohydrate into acid taking place.

The whole process, one class of organisms clearing the way for the activity of another, is termed a "*metabiotic cycle*," or "*metabiosis*." It often happens that two or more bacteria grow side by side and each assists the other, as for instance the B. tetanus, which is an anäerobic organism under ordinary circumstances, may be grown äerobically if the culture is also inoculated with B. pyocyaneus; such a phenomenon is termed *symbiosis*, and is of great importance in many pathological conditions of mixed infection.

Bacteria, as will be easily understood from the foregoing paragraphs, produce various chemical substances as the result of their growth; some of these are due simply to the splitting up of the various molecules of food stuff into simpler parts; the one is absorbed by the organism and built up into its protoplasm, the other remains in the solution. Other compounds are probably excreted by the organisms, and others again are only obtainable in any quantity from the bodies of the bacteria themselves. Some of these bacterial products are brightly coloured pigments, and it is easy to note in

examining a cultivation of such a chromogen that the pigment produced is not always confined to the actual area of growth of the organism but diffuses widely into the nutrient medium, demonstrating clearly the method in which the products of an organism taint the medium in which it grows, and in this way may check its own development (*cf.* B. pyocyaneus).

The products of vital activity of bacteria are of many kinds, the whole of which are generally now included under the term "fermentation-products."

These fermentations require considering in detail.

Production of Acid and Alkali.—The majority of bacteria produce an alkaline reaction when grown in a medium free from carbohydrate; some, *e.g.*, the diphtheria bacillus, when grown in ordinary broth containing traces of carbohydrate, for the first few days give an acid reaction; later the reaction changes to alkaline, often due to the presence of ammonia.

Very many bacteria—and of these a considerable number are mouth bacteria—are capable of fermenting carbohydrate with the production of acid. The fermentability of various carbohydrates differs widely, glucose being the most easily fermentable. Lactose is also fermented to acid by mouth bacteria. The carbohydrates of the mono-saccharide group are those most easily acted upon, the general equation of fermentation being:—

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} = \underset{\text{Lactic acid.}}{2\,(C_3H_6O_3)}.$$

The carbohydrates of the di-saccharide group, $C_{12}H_{22}O_{11}$, are first inverted to the mono-saccharide form and then fermented. Thus:

$$\text{(i.)}\quad \underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose.}}{\overset{+}{C_6H_{12}O_6}} + \underset{\text{Lævulose.}}{\overset{-}{C_6H_{12}O_6}}.$$

$$\text{(ii.)}\quad C_6H_{12}O_6 = 2\,(C_3H_6O_3).$$

The carbohydrates of the poly-saccharide group are more complex than the other two groups, and require preliminary inversion before fermentation to acid occurs. Thus:—

$$\underset{\text{Starch.}}{(C_6H_{10}O_5)^n} + H_2O = \underset{\text{Dextrin.}}{C_6H_{10}O_5} + \underset{\text{Maltose.}}{C_{12}H_{22}O_{11}}.$$

$$C_{12}H_{22}O_{11} + H_2O = 2\,(C_6H_{12}O_6).$$

$$2\,(C_6H_{12}O_6) = 4\,(C_3H_6O_3).$$

According to Brown[1] and Morris soluble starch has the formula $(C_6H_{10}O_5)_{30}$.

These equations do not express the whole of the reaction as some of the sugar is used by the bacteria themselves, and quantities of gas are often evolved during the process.

In the formation of alcohol from sugar large amounts of CO_2 are evolved, thus :—

$$C_6H_{12}O_6 = 2\,C_2H_5.OH + 2\,CO_2.$$

It has been shown by Maly[2] that proteid is not attacked to any appreciable extent as long as any carbo-hydrate remains in the solution, the organisms first attacking the carbo-hydrate, and only when this is used up is the proteid acted upon.

Gas Formation.—Many bacteria produce gas, and a well known example is found in B. coli. com., which will produce large quantities of gas even in gelatin cultures. The chief gases formed by bacteria are CO_2, N, H, CH_4, SH_2. Unless the organism be anäerobic the gas formed generally escapes undetected into the air. When anäerobic a well-marked bubble may be seen around the colonies, as in stab gelatin cultures of Bacillus tetanus. In ordinary äerobic fluid cultures the formation of gas is best demonstrated by Durham's tube, a small test tube placed in the fluid, which becomes filled with the gas and floats in the liquid.

Heat.—Some bacteria evolve a considerable amount of heat in their growth, and at times have been held responsible for spontaneous combustion of vegetable matter (hay). Some of these organisms do not thrive below 40° C., whilst they exhibit their greatest activity at 50° to 60° C. Several species have been isolated and studied. Practically they are utilised in the formation of "ensilage," the heat evolved in the anäerobic silos being due to the fermentative activity of these "thermophilic bacteria."

Nitrification.—The conversion of ammonia to nitrite and nitrate is accomplished by a number of bacteria, which are able to grow in purely inorganic material. These bacteria, which are present for the most part in the top layers of surface soil, are of great importance to the agriculturist. In artificial media a number of bacteria produce nitrates, the presence of which may be demon-

---

[1] *J. Chem. Soc.*, Lond., 1888, 610.

[2] Hermann's *Handbuch*, Bd. v. (2) S. 239.

strated by appropriate chemical means. Certain others of these nitrogen-loving organisms assist in fixing the nitrogen of the air.

Phosphorescence.—A number of bacteria, many of them developing at 0° C., produce well-marked phosphorescence. Pflüger in 1875 first demonstrated this relationship of bacteria to the silvery phosphorescence seen on unsound fish and meat. The amount of light produced has been shown to be sufficiently great to photograph small objects placed near the cultivation. The class, as a whole, show a well marked preference to certain food-stuffs; in all cases a good supply of oxygen and about 3 per cent. of salt (NaCl) are required for the production of phosphorescence.

Chromogenesis.—A considerable number of organisms, many of them belonging to the pathogenic varieties, produce various colouring matters, or pigments. The colouring matter may be confined to the bacteria themselves, or become diffused through the medium in which they grow. The pigments are often composed of several distinct chemical bodies which may be separated by chemical means. For instance B. pyocyaneus, the bacillus found in green pus, has been found to produce two if not three varieties of pigment. One may be easily obtained by extracting a cultivation with chloroform and crystallising out, when long delicate needles of a bluish-green tint are obtained, which changes to red on the addition of weak acid. The other pigment of B. pyocyaneus is a fluorescent green.

B. prodigiosus, B. rouge de Kiel, and several bacilli found in the mouth, produce a fine red pigment. Two of the pyogenic cocci, Staphylococcus pyogenes aureus, and S. pyogenes citreus, produce well-marked pigmentation; the colouring is not diffused into the culture medium. I have met with all the above bacteria in the mouth. Among other chromogens from time to time met with in the mouth Sarcina aurantiaca and Sarcina lutea are common. B. liquefasciens fluorescens, producing a fluorescent blue-green pigment, is also frequently met with. I have found this organism present in several cases of "green stain."

The chromogenic function of bacteria is considerably modified by environment. Most chromogens only produce pigment when grown at a low temperature, 20° C., and it is often possible to artificially produce a variety of non-colour-producing organisms by simply growing them at 37° C. for several generations. For some little time after the organism has been subjected to the higher

temperature it remains colourless, even when transferred to fresh tubes incubated at 22° C. In some species no colouration takes place at the low temperature; the number is, however, limited to a few isolated examples. Thus the spirillum of Metchnikoff, when grown on potato at 37° C. forms a dark chocolate coloured layer, while at 22° C. no growth takes place. The bacillus of glanders is another organism producing colouration at the higher temperature only.

The majority of chromogens require free oxygen for the elaboration of pigment, a few only producing their characteristic colour in the absence of air; among these Spirillum rubrum may be mentioned. This organism is of interest, as Hueppe managed to so modify a race that the pigment was produced äerobically.

Formation of Enzymes.—Many bodies belonging to the class of enzymes or ferments are produced by micro-organisms.

Bacterial enzymes are of two classes: (*a*) those bodies which are freely soluble and are either excreted by the bacteria, or remaining in the bacterial plasm are easily dissolved out—*inter cellular*; (*b*) those ferments which are only obtainable by trituration of the bacterial cells, and not soluble under normal conditions—*intra-cellular*.

The enzymes produced by certain bacteria digest fibrin and gelatin when in an alkaline solution. These proteolytic enzymes may be separated from a culture of an organism by shaking up with chloroform water, filtering, precipitating with absolute alcohol, filtering and taking up the residue with sat. thymol water. The resulting filtrate will liquefy gelatin if the organism treated produced a liquefying enzyme. In this way I have obtained extracts from various mouth bacteria which will digest decalcified dentine.

Enzymes induce the various changes with which they are associated by a process of hydration or hydrolysis, that is by the addition of water to the body fermented, with the ultimate cleavage of the molecule into bodies of simpler chemical composition.

These enzymes do not act well in an acid medium as do those of animal origin which react best in the presence of acid.

Another class of enzyme produced by bacteria is the ferment allied to rennet bringing about coagulation of milk.

A ferment, changing sugars of the disaccharide to the monosaccharide form, is also formed by some bacteria, a few of which occur in the mouth.

Putrefaction may be termed the fermentation of nitrogenous bodies by bacteria, and probably consists of a series of complicated changes occurring naturally by symbiosis. The first stage is a transformation of the albumin present to peptone, this being followed by the production of various gases, acids, bases and salts from the peptone.

The bad smell of putrefying animal matter owes its origin to several members of the aromatic series, among which are *indole* and *skatole*, or β. methyl indole. Indole combines with nitrous acid to form a red compound (nitroso-indole). Use is made of this in determining the presence of indole in a cultivation. Those bacteria (*e.g.*, cholera vibrio) which produce nitrite plus indole give the red colour on the addition of *nitrite-free* sulphuric acid. If no nitrite is formed, as B. coli, nitrite must be also added, either in a 0·3 per cent. solution of potassium or sodium nitrite, or by adding yellow (commercial) nitric acid, containing nitrites. This indole reaction without the addition of nitrite is often known as the cholera red reaction, as it was first described in connection with that organism.

Phenol, ortho- and para-cresol, leucine and tyrosine are also formed by the action of putrefactive bacteria.

Sulphuretted hydrogen is commonly formed among other gases, and is recognised by adding an iron compound (iron-lactate) to the culture medium.

Ptomaines.—Various poisons are formed by the decomposition of putrefying albuminoids, some of which produce serious symptoms when ingested by man. The substances often result from the growth of organisms in various articles of food, among which may be mentioned *tyrotoxican* from the decomposition of cheese, and *hydrocollidine* from the flesh of cattle.[1]

It occasionally happens that putrefaction with the formation of similar poisonous bodies may go on in the intestinal canal, the products in such case receiving the term *leucomaines*, and are probably the chief cause of the headaches so often associated with constipation.

Many putrefactive bacteria are obtainable from the mouth; especially is this the case in individuals possessing unclean mouths and many decomposing roots.

The ptomaines are definite chemical bodies which have been

---

[1] Vaughan and Noug, "Ptomaines and Leucomaines."

isolated by Briger and others and their percentage composition determined. The toxines (see below) have not yet been isolated in a chemically pure form.

Toxines.—A number of pathogenic bacteria produce poisons during the period of growth, and the symptoms of certain diseases are due to the absorption of these toxines. These bodies have not yet been isolated in a true chemical form, but by filtering a broth cultivation in which a toxine-forming organism has been grown the bacteria are filtered off, and the germ-free filtrate contains the toxines. This solution of toxine injected into susceptible animals produces death. The filter used is of unglazed porcelain (see fig. 11). By evaporating the filtrate to one-third at 30° C. *in vacuo*, precipitating by alcohol, again taking up in water, and repeating the process several times, a white powder may be obtained from the filtered culture of diphtheria. This white powder injected into guinea pigs produces the same symptoms as injection of living diphtheria bacilli.

Sidney Martin[1] isolated from the cultivations of diphtheria, as well as from the spleen, &c., of patients dead of the disease, two bodies, one of the nature of an albumose, the other an acid. These substances when injected into animals produced the same symptoms as diphtheria toxine. There was, however, a certain difference due to the fact that the albumose isolated behaved as a digestive enzyme, forming the true toxine from the body tissues. Smaller but repeated doses produced more marked effect than single large doses; the natural conditions of diphtheria poisoning, consisting of gradual absorption rather than sudden intoxication, were thus copied.

Toxic bodies may be prepared from cultivations of tetanus, typhoid, staphylococcus aureus, cholera, &c., by the method adopted for diphtheria toxine.

The fluids obtained by filtration, &c., or the amorphous alcohol precipitate both show similar reactions, that is: (*a*) on injection into animals; (*b*) a temperature of 58° C. for two hours destroys the pathogenic properties. These toxines are classed under the group of intercellular toxines, destruction of the bacterial cells not being necessary to obtain the poisons, which are freely soluble in the liquid media in which the bacteria are grown. On the other hand many organisms, if not all, possess poisonous properties

---

[1] Local Government Board Reports, 1891.

within their own plasm or micro-protein. The material used in "vaccination" for typhoid fever consists of such intra-cellular poison of the typhoid bacilli.

The inter-cellular and more soluble toxines appear nearly related to the digestive enzymes of animal glands, such as trypsine and pepsine in their method of action, and it is extremely probable that the nerve degeneration of diphtheria and the solution of fibrin by digestive ferment proceed along exactly comparable lines. Under such an hypothesis it is easy to understand why a small continued dosage of a given bacterial poison will produce such profound effect, and how it comes about that such minute quantities are relatively so potent. Probably the change is the same process of hydration that we have seen occurs in the carbohydrate transformation, and that when the molecule has become enlarged by the addition of water to a given extent it breaks up along new planes of cleavage.

It is of course possible that the true toxic bodies are definite chemical compounds which are precipitated along with the albumoses in the alcohol method adopted. So far, however, all attempts to obtain definite crystalline bodies have failed, and all we are able to state is that the toxine, whatever it may be, is found in the precipitate thrown down by alcohol from cultivations of bacteria-forming toxines, and that the precipitate thus found certainly contains albumoses.

For further information on toxines see chapter on immunity.

CHAPTER III.

Sterilization and Disinfection.

Bacteria are the most widely distributed of living things; they teem in the dust of cities, in hospital wards, they are to be found in countless numbers in the soil, in the air we breathe, in common articles of food, in water, and particularly in the dusty air of streets and living rooms.

The air of high mountains and mid-ocean are generally practically free from organisms, whereas city air may contain as many as 100,000 or more per cubic foot. They have recently been found in glacier ice.

The organisms present in air are by no means all pathogenic, but at the same time many pathogenic bacteria are frequently present; amongst them the pyogenic cocci are common. The source of the organisms in the air is for the most part dust, and where dust contains the dried expectoration of tuberculous persons the tubercle bacillus is invariably present. During damp and wet weather the number of organisms present diminishes considerably, the falling rain freeing the air from suspended matter and bacteria, which are carried away with the surface water in properly drained places, or remain in the mud of pools to be wafted into the air as dust when the water evaporates.

Bacteria of the air are, for the most part, simple saprophytes, and although not disease-producers in the ordinary way are capable of setting up profound changes in organic fluid exposed to their advent, producing "disease" in such articles as milk, meat, &c. Many of the spores of the higher fungi are air-borne as well as yeasts and torula. A gelatin plate exposed to the air for a few moments will generally develop a number of colonies when incubated. I have already referred to the minute size of these micro-organisms, and it is not difficult to understand that almost anything with which we commonly have to deal in bacteriological work is contaminated

with numbers of unseen organisms ready to develop the moment we make the conditions favourable for them. And what is true of bacteriological apparatus is still more true of dental and other instruments, for with these latter not only are air-borne organisms present, but also those from infected wounds, oral secretions and decaying dentine, septic pulps, &c., with which they have been in contact.

Owing to their minute size bacteria are carried about by the slightest currents and motion of the air, but in still air they gradually sink to the lower strata. Tyndall proved that when the dust in a specially constructed room had been allowed to settle till the polariscope showed no trace of suspended matter, sterile open vessels of nutrient solution could be freely exposed without decomposition taking place as long as the dust remained quiescent. When, however, the dust was again made to rise the fluids quickly became putrescent.

It is evident that any materials with which we wish to conduct bacteriological experiments must be first of all freed from the organisms naturally present, otherwise we shall be unable to determine if the particular fermentation or growth we are examining is the product of a single species or of a mixture of species, or in mycological parlance know if we are dealing with *a pure culture.*

Pure cultures, consisting of members of one given species only, are the means by which determinative bacteriology has been rendered possible. Although much of the earlier work was conducted with what are now known to have been mixtures, it must not be supposed that the combined action or symbiosis of bacteria is to be disregarded, many of the most interesting of natural fermentations belonging to symbiotic phenomena.

But to properly study the combined activity of two or more bacteria we must first have pure cultures of each from which to make our mixture. A considerable portion of bacteriological technique is directed towards obtaining pure cultures, and the process of excluding adventitious organisms is termed *sterilization.* Heat in some form is commonly used, of such a temperature that the articles sterilized are not injured while the organisms present are killed. The difference in resisting power has been already noted, and the reader is advised to take particular note of the "resisting power" of various organisms, as it has large practical bearing upon the question of sterilization.

Heat is applied in two forms : (1) Dry heat ; (2) Moist heat.

(1) **Sterilization by Hot Air.**—The various pieces of apparatus used in bacteriological work, such as flasks, test tubes, Petri dishes, and the like, are sterilized by heating to 150° C. for three-quarters

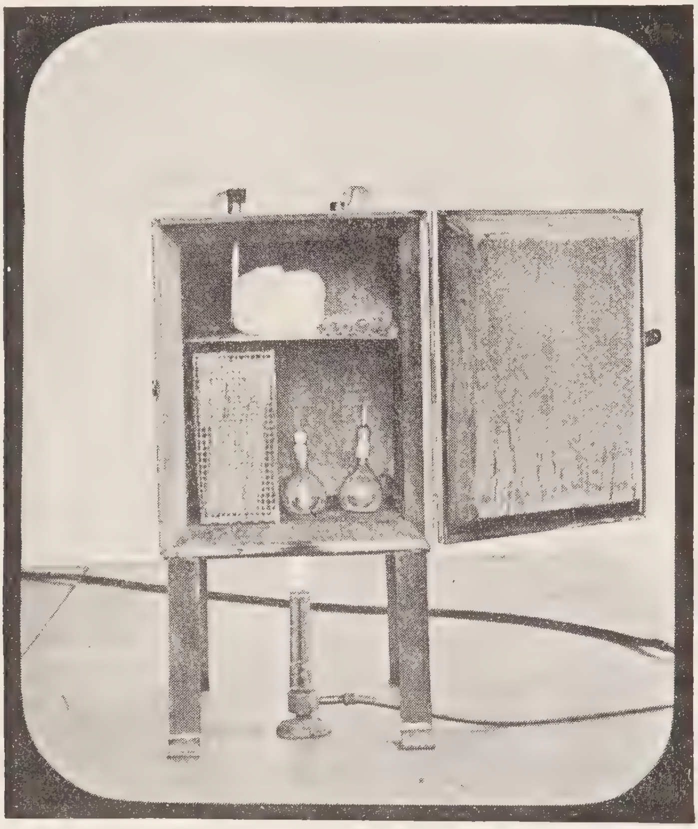

Fig. 4.—Hot-Air Sterilizer with Apparatus ready for Sterilization.

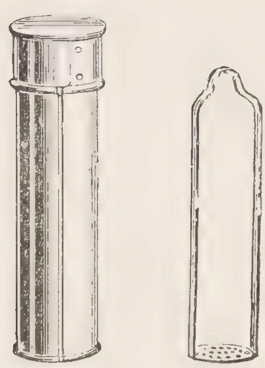

Fig. 5.—Copper Box and Rack for Sterilizing Petri Dishes and Capsules.

of an hour in a hot-air sterilizer ; the flasks, &c., are first plugged with cotton wool plugs, which so long as they remain dry prevent the passage of bacteria. The hot-air sterilizer (fig. 4) consists of a

copper or sheet iron box with hollow walls and a fire-brick bottom, placed upon a stand to admit of a large gas burner underneath. There is a hinged door opening the whole width of the sterilizer. In the roof are two tubes communicating with the inner chamber, through which a thermometer is placed to register the temperature of the interior.

Petri dishes, capsules (figs. 6 and 7) small Petri dishes, 5 cm. wide), pipettes, &c., are placed in special copper boxes with a central movable rack from which the plates may be lifted out when required (fig. 5).

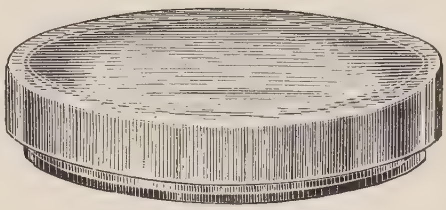

FIG. 6.—PETRI DISH.

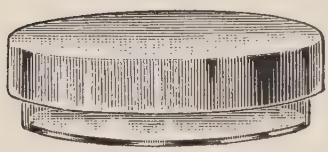

FIG. 7.—GLASS CAPSULE.

Test tubes, flasks, &c., are first plugged with cotton-wool. A piece of wool is folded up and twisted into a firm plug and forced into the mouth of the tube, about a third left projecting. The test-tubes are placed in wire crates which fit into the sterilizer.

A convenient addition is a "contact alarm," so arranged that a bell rings when the temperature reaches the point required.

The temperature is allowed to rise slowly to 170° C., when the gas is turned out and the apparatus allowed to cool down. The door must not be opened till the temperature has fallen to 60° C.

The temperature here suggested is that which is found to destroy spores, the vegetative forms succumbing at a much lower temperature (68° C.). For the various liquid and solid media used 170° C. is too high, and would evaporate and char the tube contents. Streaming steam in the steam sterilizer is therefore used.

(2) **Sterilization by Streaming Steam.**—Although the spores of most bacteria resist the application of 100° C. for a considerable

time, yet the vegetative forms are destroyed at relatively low temperatures. Having this in mind Tyndall suggested the discontinuous method of sterilization by streaming steam. The operation is generally carried out in a Koch's or other steam sterilizer. Tyndall found that although the spores were not killed by twenty minutes' steaming the vegetative forms were, and therefore if twenty-four hours were allowed to elapse after the first heating, any spores present would germinate in the nutrient media, and be easily destroyed by a subsequent heating. This is the method generally

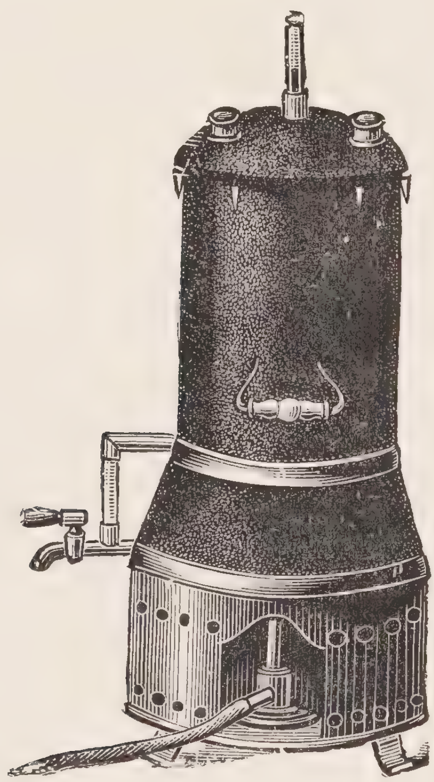

FIG. 8.—KOCH'S STEAM STERILIZER.

adopted. The media is placed in the test tubes which have already been sterilized in the hot-air sterilizer and placed in the steamer for twenty minutes on three successive days, after which the media is ready for use.

The steam sterilizer (fig. 8) is simply a modified potato steamer or double saucepan, with an asbestos jacket to minimise radiation. The tubes should not be placed in the apparatus until steam is given off, otherwise considerable condensation takes place, and for the same reason should be removed as soon as sterilized.

(3) **Steam under Pressure** is also made use of in various ways, and is the method generally adopted for the disinfection of articles of clothing, bedding, &c. The autoclave (fig. 9) is the apparatus used in the laboratory, and consists of a strong copper boiler with removable lid, which can be adjusted by means of a series of thumb screws set at intervals. There is a pressure gauge, thermometer well, and safety valve in the lid.

Water requires a pressure of 15 lbs. to the square inch to boil at 100° C., and 15 lbs. extra, that is, two atmospheres, to boil at 115° C. The safety valve is set to blow off at 115°, and the medium sterilized for fifteen minutes at this temperature.

Gelatin must *not* be sterilised in the autoclave as a considerable amount of hydration to gelatin peptone often occurs, impairing the

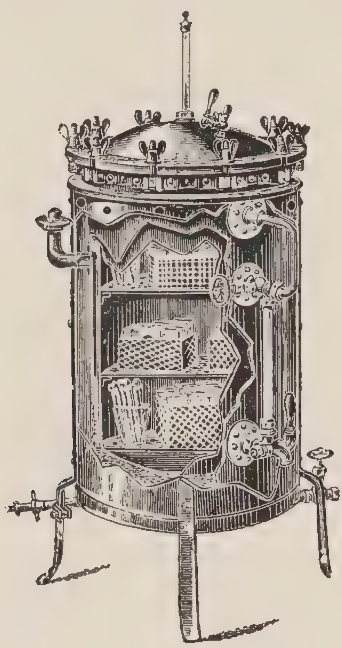

FIG. 9.—AUTOCLAVE.

value of the medium, which may subsequently refuse to set; even when the temperature does not rise above 105° C. peptonisation will occur, and long boiling may also produce the same effect. The peptonisation of gelatin by autoclave sterilization was particularly impressed upon my mind when attempting to sterilize gelatin in bulk for some physiological experiments; two litres of 20 per cent. gelatin were placed in the autoclave at 115° for twenty minutes, but on cooling to the ordinary room temperature afterwards the whole quantity refused to solidify—the gelatin was entirely peptonised. Milk may be conveniently sterilized in this way; agar generally darkens considerably, and had better be sterilized by the

discontinuous method. Broth may be autoclaved, as may potato, but all these media are best sterilized by streaming steam.

(4) **Sterilization at Low Temperatures.**—The vegetative forms of most bacteria are easily destroyed at low temperatures (55°—60° C.). Advantage is taken of this fact in sterilizing blood serum and other fluids which coagulate at 75°—100° C. The serum collected under aseptic precautions is kept at a temperature of 57°—58° C. for an hour on five or six successive days. The temperature adopted does not coagulate the serum, which may be used in the fluid condition if desired, and more important still may be kept in sterile flasks of convenient size for indefinite periods, care being taken to avoid certain infection of the plugs with air-borne spores. Roux's paper caps may be advantageously used, or better still "sterilized milk" bottles or rubber caps may be placed on the tubes.

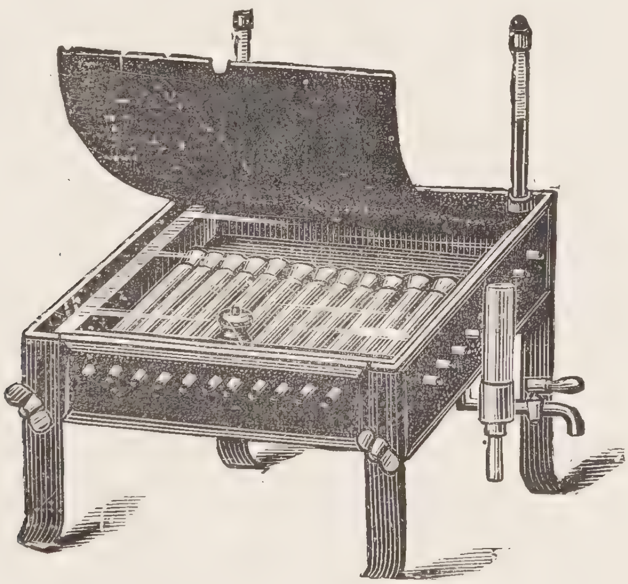

FIG. 10.—KOCH'S BLOOD SERUM INSPISSATOR.

Sterilization of Instruments.—The various instruments used for *post-mortem* examinations, injection syringes, &c., are sterilized by boiling in water in a suitable copper vessel fitted with a perforated tray. A small quantity of carbonate of soda is added to the water to prevent rusting. A quarter of an hour is generally con-considered sufficient exposure to boiling water for all practical purposes.

Dressings, bandages, and the like, may be sterilized in the autoclave, or by the hot air method; the former is preferable, dry air being afterwards passed through the apparatus.

The platinum wires used in the inoculation of media during the process of making cultivations, forceps and various other small articles that are not injured by heat are sterilized by heating in the bunsen flame. The platinum or platino-iridium inoculating needles must be heated in the flame till red hot before and after use, to prevent the contamination of the culture or the dissemination of the organisms in the culture tube. The platinum needle is first heated to redness and then the glass or aluminium handle passed through the flame also. It is essential that no wire should be laid down under any consideration whatever without previous sterilization.

It is not perhaps out of place to note here that the majority of dental instruments may be sterilized by boiling with water containing 1 per cent. of sodium carbonate, in the manner adopted for other surgical instruments.

With some of the finer instruments it is better to substitute pure almond oil for the water in a small sterilizer; the edge and temper are not affected in the least.

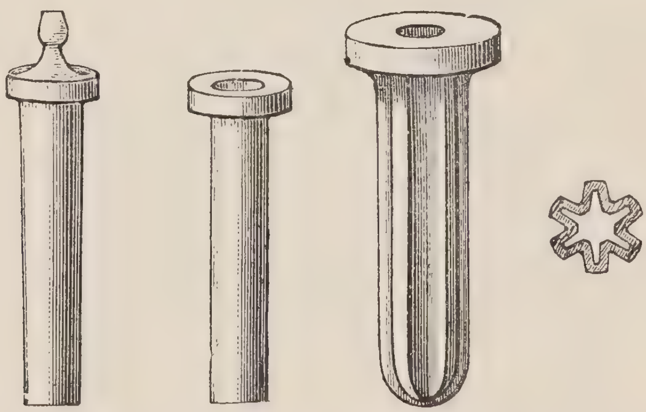

Fig. 11.—Unglazed Porcelain Filters (Pasteur-Chamberland).

Sterilization by Filtration.—This method is largely adopted in the preparation of the soluble products of bacterial activity, such as toxines and enzymes.

The material, broth cultures, for instance, is placed in a specially constructed hollow cylinder of unglazed porcelain (fig. 11). The cylinder is fitted into the mouth of a sterile filter flask by means of an india-rubber washer previously sterilized by boiling, and negative pressure developed by means of a filter pump. The canals of the porcelain are so minute and tortuous that the fluid alone can pass through, the bacteria being arrested. To sterilize the "filter candle" after use it may be heated to redness in a muffle, using great

caution, or hot alkaline permanganate solution may be filtered through, by which means the bacteria remaining in the canals of the filter are dissolved. Hydrochloric acid may also be used, but considerable care is required to wash away the acid afterwards.

Many fluids may be prepared for cultural purposes by filtration in this way if the process of sterilization at 100° C. damages them.

Various forms of water filters are constructed of unglazed porcelain and form the only efficient bacteriological filters. These filters do not however work indefinitely, as in about a week the bacteria which are arrested by the windings of the canals have grown to such an extent that the filtrate becomes contaminated.

ANTISEPTICS AND DISINFECTANTS.

The terms antiseptic and disinfectant are somewhat misleading in that a substance which will certainly destroy bacteria or their spores in a fairly strong solution (disinfectant) will only inhibit their growth when used in higher dilutions (antiseptic). It follows then that antiseptics only hinder the growth, while disinfectants destroy the life, of bacteria.

A large number of chemical substances have been used from time to time, many of these substances eventually proving to be of little value, not perhaps so much on account of the inefficiency of the chemical to destroy bacteria as on account of the wasting of the disinfectant by reason of other substances present. It may often happen that owing to the presence of some body with which the disinfectant easily forms compounds—such for example as mercuric chloride and albumin—a large quantity of the supposed disinfectant is rendered inert by precipitation. Permanganate of potash readily oxidises all organic matter, whether bacteria or proteid compounds; it is therefore necessary in choosing an antiseptic to obtain the most efficient one for the special purpose for which it is to be used, having regard to the particular local conditions. It must also be remembered that many antiseptics and disinfectants are at least as injurious to the cells of the body as to the bacteria they are employed to destroy, and a solution used in such strength actually favours the entrance of the organisms by lowering the tissue vitality. This effect of antiseptic solutions is often overlooked, and it follows that a great deal more may be done by preventing the access of organisms than by attempting to destroy them when they have once gained a footing.

The following list of antiseptics and disinfectants gives some of the more common ones in use with their relative strength as determined practically by laboratory experiments. These tests, however, are more favourable to the antiseptic used than the organism tested, which is growing artificially and not in its usual habitat. The use of spores and their death as determined by absence of germination is more reliable; the spores to be tested are dried upon sterile silk threads and immersed for various periods of time in the antiseptic to be tested, then washed with boiled distilled water to remove traces of antiseptic and transferred to a culture tube.

Another method is to add various quantities of the antiseptic under investigation to broth cultivations of the organisms experimented with; in this case care must be taken to avoid fallacies due to the neutralisation of the antiseptic by the medium used. The cultivations may be either fully developed ones, or one or more loop-fuls (öse) of culture may be inoculated into the nutrient medium containing the antiseptic to be tested. In making the subsequent sub-cultivations to test the destruction or inhibition of the organisms care must be taken to use a sufficiently large quantity of nutrient medium, otherwise the amount of antiseptic in the öse may invalidate the result. Control tubes should invariably be made.

Sternberg recommends mixing the standard culture and the diluted antiseptic in equal proportions; thus 10 cc. of sterile broth containing 1 in 200 carbolic is added to 10 cc. of a twenty-four hours' broth culture of the given organism (= 1 in 400); plate cultivations are then made at given intervals.

Many substances, such as concentrated solutions of sugar or common salt, prevent the development of bacteria but do not kill them.

Disinfection ôf Hands, &c.—The bacterial flora of the skin is of a varied nature, and owing to the cracks and fissures of the epidermis, particularly the hands, it is difficult to remove the bacteria; moreover the bacteria actually live upon the dead epithelial cells, rarely however penetrating the true skin. The best method to adopt is first thorough scrubbing with a nail brush (boiled and kept in 2 per cent. lysol), soap and hot water, to remove as much of the dry epidermal scales as possible. The hand should be then soaked for two minutes in some antiseptic solution such as the one recommended by Lockwood[1], 1 of biniodide of mer-

---

[1] *Brit. Med. Jour.*, Jan. 11, 1896.

cury in 500 of methylated spirit, which is subsequently washed off in 1 in 3,000 biniodide solution. This treatment does not cause the same roughness that mercuric chloride or carbolic so often produces. It need hardly be added that unless a perfectly sterile towel is used the disinfection is of no avail, and in operative surgery they are discarded entirely, and should not be used by the dental surgeon to dry his hands before the operation of extraction.

The following short list of antiseptics and disinfectants gives the most useful; for other and extensive lists the reader is referred to Sternberg's "Bacteriology," Macfarland's "Pathogenic Bacteria," and Hueppe's exhaustive article in "Principles of Bacteriology."

Formalin (40 per cent. sol. of formic aldehyde gas in water) ...	1 in 40,000
Biniodide of Mercury	1 ,, 35,000
Bichloride of Mercury	1 ,, 14,000
Lysol	1 ,, 1,000
Carbolic Acid	1 ,, 133

These figures give the relative strengths of solutions which will restrain the growths of bacteria but will not always destroy them.

Salicylic acid and quinine are also powerful antiseptics, whilst iodoform, so commonly used, must be first changed into iodine—a somewhat rare thing—before it is effective. Its chief action is the neutralisation of the products of the organisms. The various mineral acids are strong disinfectants, and 5 per cent. HCl added to mercuric chloride greatly increases its efficiency. Lysol, consisting of coal tar oil, phenol and soap, is advantageous in that it is strongly alkaline and dissolves grease. It is used largely in general laboratory routine, especially for soaking used slides, the coverslips becoming detached by solution of the balsam as soap. A jar containing a 2 per cent. solution should be kept on the laboratory bench.

CHAPTER IV.

Methods of Observing Bacteria—Microscopical.

THE microscopic examination of bacteria is carried out in two ways: (1) observation of living organisms; (2) stained preparations.

(1) **Observations on Living Bacteria.**—A small tin ring is cemented on to a glass slide with canada balsam, forming what is known as a hanging-drop slide (fig. 13). A clean coverglass (see

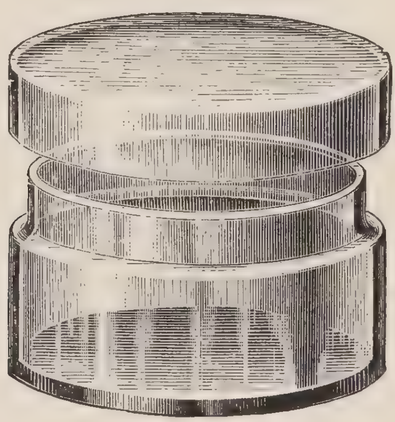

FIG. 12.—COVERGLASS JAR FOR KEEPING COVERSLIPS IN ALCOHOL.

appendix) is removed from the jar (fig. 12) and the alcohol burnt off; a drop of water placed in the centre by means of the platinum loop, and the drop inoculated with a minute amount of the culture to be examined; the ring on the hanging drop slide is smeared with a little vaseline by means of a small paint brush, and the coverslip placed upon it drop downwards; the vaseline prevents the coverslip falling off and keeps the preparation from evaporating. The preparation is now ready for examination, and is placed under the microscope and examined first with the $\frac{1}{6}$. Motility, Brownian movement and spores should be looked for; the spores, if present, appear as highly refractile bright dots.

To watch the development of an organism under the microscope some sort of warm stage is required, a constant temperature being maintained by means of circulating water and a thermoregulator. The hanging drop is used, with a nutrient solution substituted for the water.

The hanging-drop method is used for the determination of the agglutinating power of serum, as in Durham's (Wedl's) typhoid reaction and spore germination, chemiotaxis, and many other experiments with living organisms. Every organism should be submitted to this process besides the methods of staining given below.

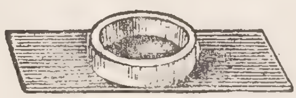

FIG. 13.—HANGING DROP SLIDE.

(2) **Coverslip Preparations.**—In coverslip preparations the bacteria are fixed on the coverslip, and stained by one or other of the various stains given below.

A coverslip is taken, and when the alcohol has been removed, a drop of sterile water is placed in the centre. With a sterilised platinum wire, sterilised by heating to redness in the flame, a small quantity of the culture to be examined is removed and added to the drop of water. Only a small amount is used, otherwise the preparation is too thick and the individual organisms massed together in clumps. A faint cloud throughout the drop is all that is required. The drop now containing the bacteria is carefully spread over the surface of the coverglass and allowed to dry.

When dry, *but not before*, the coverslip is "flamed." To do this take the coverslip between the finger and thumb and pass downwards through a bunsen flame, repeating the process three times. There is nothing mystic in the "three times through the flame," but it has been found by the experience of a large number of workers that this fixes the bacteria properly without damaging them for staining afterwards. After a little practice the student will be able to hold the coverslip in forceps, but it is far better to use the fingers at first until the method is mastered. After cooling the coverslip is flooded with stain. A good method is to use an

indiarubber coin pad such as is used for "change" in many shops; the slips can be easily manipulated in this way. In some processes it is better to immerse the coverslip in a watchglass full of stain.

When stained the preparation is well washed in water, dried between folds of blotting paper, and may be finally dried a safe distance above the flame (one foot). The preparation is then laid film upwards upon a piece of blotting paper, a drop of canada balsam dissolved in xylol placed in the centre, and a clean slide pressed upon it; the blotting paper absorbs any excess of balsam. The preparation is now ready for microscopic examination. A drop of cedar oil is placed on the coverslip and the oil immersion lens lowered until it touches the oil, and all but touches the glass; great care must be exercised to prevent the lens actually coming in contact with the glass, otherwise it may be irreparably damaged. To find the focus, rack upwards with the coarse adjustment until the film comes into view and then use the fine adjustment.

Films from Liquid Cultures.—The films made from broth or from the mouth direct contain a considerable amount of material which stains as well as the bacteria, forming an undesirable background. To prevent this the film must be "cleared" with some solution which does not interfere with the later processes of staining.

The films may be cleared in 1 per cent. acetic acid, or in absolute alcohol.

Another method of fixing film preparations suggested by Goulard is as follows: the coverslips, dried in air but not flamed, are immersed in a solution of absolute alcohol 25 ccm., pure ether 25 ccm., alcoholic solution of mercuric chloride 20 per cent. 0·5 ccm.

The films are left in for five minutes or longer, washed well in water and stained.

Tissue Preparations.—These preparations may be fresh, the tissue being cut with the freezing microtome, or fixed and hardened, and cut with the rocking or other microtome.

Fixation.—Small pieces of tissue may be hardened and fixed at the same time in absolute alcohol. Corrosive sublimate, a saturated solution in 0·75 per cent. sodium chloride solution is very useful; pieces $\frac{1}{8}$ in. in size or less are left in solution for twelve hours, larger pieces for a longer time. After fixing they are placed in a gauze bag in running water for twenty-four hours, and then passed through three percentages of spirit, 30 per cent., 60 per cent., 90 per cent.

Some iodine is added to the 60 per cent. to remove the last traces of the mercury salt.

Embedding.—The various stages in the process are as follows:—

(1) Preliminary fixation and hardening as above.

(2) Absolute alcohol to complete dehydration.

(3) Absolute alcohol and xylol, equal parts, for twenty-four hours.

(4) Xylol twenty-four hours.

(5) Xylol and paraffin twenty-four hours in paraffin bath.

(6) Pure paraffin three or four hours.

(7) Melted paraffin poured into mould composed of two L-shaped pieces of brass, and just before it sets the tissue placed in it.

(8) Trim up when hard and cut on microtome.

Preparation of Sections of Teeth to show Bacteria in situ.—Owing to the leathery consistence of dentine when decalcified, the specimens cannot be cut in paraffin with any degree of success, and celloidin is generally employed.

Care must be taken in decalcifying, otherwise the bacteria do not stain well; the best agent is trichloracetic acid, suggested to me by Dr. Spriggs, which gives admirable results.

The tissue is fixed in Goulard's solution or other fixative, washed and transferred to the acid (5 per cent. solution) till soft.

When thoroughly softened the preparation is well washed, dehydrated and embedded in celloidin in the usual manner. The sections are stained after cutting. Carious dentine, &c., may be embedded in gum while fresh and cut when frozen.

To prepare and stain the sections obtained by the paraffin method the embedding process is reversed.

Float the section in warm water on to a clean slide and dry. Remove the xylol with absolute alcohol, the alcohol with water.

The specimen is now stained, washed rapidly in water, then alcohol, and finally xylol, and mounted in Canada balsam dissolved in xylol.

Blood Films.—*Method I.*—Place a drop of the blood to be examined upon a clean slide near one end. Take a second slide and place the edge in the drop so that the whole of it becomes wetted, then push the slide along the surface of the first, keeping the second slide inclined.

Method II.—Moisten the edge of a cigarette paper with the blood to be examined and quickly smear the slide or coverslip. Two or more coverslips may be held in a clip or on a piece of blotting paper by means of a slide.

These films require special treatment if the corpuscles are to be preserved. The films are fixed by one of the following methods :—

(1) In a hot air oven at 120° C. for an hour.

(2) In equal parts of alcohol and ether for half an hour.

(3) In saturated mercuric chloride solution for three to ten minutes.

After fixation the films are washed, stained, dried and mounted.

For special stains for blood films the reader is referred to the large text-books.

METHODS OF STAINING BACTERIA.

General Principles.—The stains generally used in the laboratory for the staining of bacteria belong to the aniline series of basic nature, the bacterial plasm staining much in the same way as the nuclear chromatin of animal cells.

The aniline dyes are divisible into two series according to whether the acid or basic part of the dye is concerned in the process of staining. The basic stains are the ones that have the greatest affinity for the nuclear chromatin and bacteria, the acid for the protoplasm of the cell.

The following aniline dyes are among the ones commonly used in mycological work : Aniline gentian violet ; dahlia (methyl-violet) ; methylene blue (phenylene blue) ; methyl green ; thionin blue ; Bismarck brown (vesuvin) ; fuchsin (basic rubin).

General Remarks on Stains.—The red and violet stains are the most intense in their action, and it is particularly easy to overstain with them ; they are also liable to form "background." Specimens stained with gentian violet may often be cleared in absolute alcohol without decolourising the organisms.

The two blue stains are not so intense in their action but give more detail of structure ; methylene blue particularly is useful in this respect, many appearances of the protoplasmic contents of the organisms being only demonstrable by its use. They are largely used for general routine work and for counterstaining for contrast.

Stock solutions of the above stains are conveniently kept in alcohol ; a quantity of the stain is placed in a glass-stoppered bottle and alcohol poured in, as it is used from time to time the bottle is filled up with fresh alcohol.

Watery solutions of the stains are also used and may be kept made up, 1 per cent. being the usual percentage.

All stains require filtering, as decomposition occurs with the formation of granules which become deposited on the specimen; the violet and red stains are particularly liable to do this.

Many, if not most, stains work better and with more rapidity if some mordant be also added to the solution; among these mordants, carbolic acid, aniline oil, and caustic potash are severally employed. Carbolic is the mordant commonly used; a 5 per cent. watery solution is kept made up and only mixed with the alcoholic solution of dye immediately before use.

In some staining processes decolourising agents are employed to differentiate between certain bacteria, some remaining unaffected, others are decolourised (Gram's method).

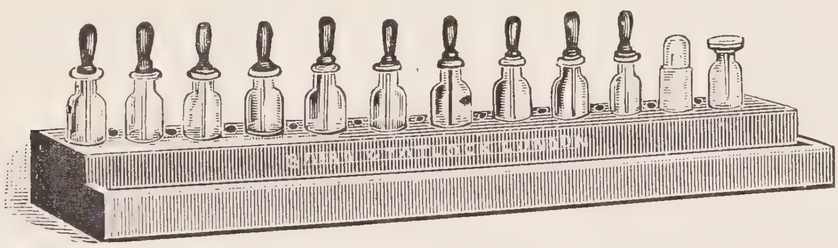

FIG. 14.—STAND TO HOLD BOTTLES OF STAINS, &c., FOR LABORATORY BENCH.

Formulæ of Stains.

(1) *Löffler's Methylene Blue.*

Saturated alcoholic solution methylene blue ..	30 cc.
Potassium hydrate (1 in 10,000 in distilled water) ..	100 cc.

Very little liable to overstain, even when left in contact for a long time. Eosin may be used as a counter-stain.

(2) *Carbolic Methylene Blue.*

Methylene blue	1·5 gm.
Absolute alcohol	10 cc.
Carbolic acid solution (1 in 20)	100 cc.

Carbolic Thionin Blue.

Thionin blue	2 gm.
Carbolic acid solution (1 in 20)	100 cc.

(3) *Aniline Gentian Violet.*

(i.) Saturated alcoholic solution of gentian violet.
(ii.) Saturated watery aniline oil (aniline water).

The aniline water requires filtering, and should be freshly prepared each time by shaking up 5 cc. aniline oil with 200 cc. distilled water. Immediately before use 1 part of the stain is added to 10 parts of the aniline water.

(4) *Carbol-Fuchsin.*

Fuchsin	1 gm.
Absolute alcohol	10 cc.
Carbolic solution (1 in 20)	100 cc.

With both aniline gentian-violet and with carbol-fuchsin, methylated spirit should be used to clear for one minute.

These four stains are of general application, and although by no means all that are used by bacteriologists are sufficient for most purposes. Various special methods will now be described, and the composition of the stains given under the various headings.

Gram's Method.—Solutions :

(1) Aniline gentian-violet.
(2) Iodine, 1 gm. ; pot. iod., 2 gm. ; distilled water, 300 cc.
(3) Absolute alcohol or rectified spirit.
(4) Xylol.
(5) Balsam in xylol.

Method :—

(1) Stain the section of tissue or coverslip film for five minutes, preferably by floating on the stain in a watch glass.

(2) Place in iodine solution, after washing off excess of stain, till the colour has changed to purple black ; time required one-half to two minutes.

(3) Decolourise in absolute alcohol till no more colour can be extracted.

(4) Wash well in xylol, dry and mount in canada balsam dissolved in xylol.

All bacteria do not stain by this method, and it is therefore used as a means of differentiating certain species.

There are various modifications of the process ; two only will be mentioned. (*a*) Weigert, who uses aniline oil as the decolourising agent after the iodine solution, instead of alcohol. (*b*) Muir and Ritchie, who use carbolic instead of the aniline water (1), and substituting olive-oil for the xylol (4), afterwards washing in xylol. Contrast stains may be employed with this method, and have the advantage of showing any bacteria present that have decolourised by the Gram process.

Safranin and Bismarck brown, saturated alcoholic solution diluted with an equal bulk of distilled water, give good results.

Ziehl-Neelsen method for acid-fast bacteria (tubercle, leprosy, &c.). The tubercle bacillus does not stain well with the ordinary methods

adopted for bacteria generally, but requires an energetic stain plus the application of heat.

Solutions :—

(1) Carbol-fuchsin stain.
(2) 25 per cent. of pure sulphuric acid.
(3) Alcohol, absolute or rectified.
(4) Carbol-methylene blue stain.

Method :—

(1) Stain the films for five or ten minutes in diluted carbol-fuchsin (1 in 3 water) kept hot over a water bath. The stain should steam but not boil.

(2) Decolourise with rectified spirit until no more colour is extracted, and wash in water.

(3) Plunge into the sulphuric acid solution and wash in water; repeat the process if there is more than a faint pink tinge on washing.

(4) Stain for half a minute in diluted carbol-methylene blue. Wash, dry, mount. The tubercle bacilli are stained a bright red, the background of epithelial cells and other bacteria, blue.

Spore Staining.—Solutions:

(1) Carbol-fuchsin.
(2) 5 per cent. sulphuric acid.

Method I.

(1) Stain the film as for tubercle bacilli.
(2) Decolourise rapidly in 5 per cent. sulphuric acid and wash.
(3) Counterstain with carbol-methylene blue.
(4) Wash, dry, and mount.

The spores are stained a bright red, the bacilli blue.

Möller's method of spore-staining is of considerable advantage in some cases:—

Method II.

(1) Chloroform two minutes.
(2) 5 per cent. chromic acid two minutes.

The remaining steps are similar to Method I.

In all the above preparations the film is prepared as directed on page 41. To simply demonstrate the presence of spores without attempting to stain the organisms the flaming is increased. This makes the spore envelope more permeable to the stain, although the

bacilli themselves are broken up and easily decolourise in the acid solution.

Method III.

(1) Pass coverslip fifteen times through flame. Proceed as in I., but do not counter-stain, as the bacilli are destroyed by the heating.

Capsule Staining.—Some organisms, as the pneumococcus, are possessed of gelatinous capsules, which may be stained by special processes.

Method I.

Solutions: Glacial acetic acid; aniline gentian-violet; sodium chloride 2 per cent.

(1) Immerse in acetic acid while film is wet for three seconds.
(2) Wash off acid with aniline gentian violet.
(3) Wash in 2 per cent. sodium chloride.
(4) Examine in sodium chloride solution.

FIG. 15.—BOSTON'S FORCEPS FOR HOLDING COVERSLIPS DURING STAINING.

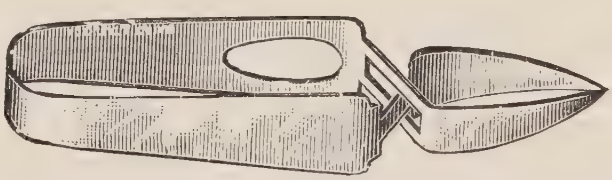

FIG. 16.—CORNET'S FORCEPS FOR HOLDING COVERSLIPS DURING STAINING.

MacConkey's: Method II.

Stain:	Dahlia	1·5 gm.
	Methyl-green (00 crystals)	0·5 gm.
	Sat. alcoholic fuchsin	10·0 cc.
	Distilled water	200 cc.

(1) Prepare film in ordinary way.
(2) Flood coverslip with stain holding it in spring forceps (Cornet's).
(3) Heat till steam is given off, and allow to remain five minutes.
(4) Wash, dry, and mount.

The cocci are stained a deep violet, the capsules a faint violet.

Flagella.—Flagella staining is one of the most difficult of all bacteriological operations, requiring a good deal of practice and not a little patience. The flagella are invisible under ordinary circumstances, and it is probable that in the process of staining they become swollen and so come into view. Another idea is that the stain is deposited upon and not in the flagella, as is the case in the ordinary staining of bacteria. It is most difficult to get both flagella and bacilli stained and the beginner must be prepared for a good many failures before a successful preparation is obtained.

In staining flagella the greatest care must be exercised that the coverglasses used are perfectly clean, that the films are not too thick, that the organisms are well separated in the emulsion used, that the flagella are not destroyed in flaming, and that the stains used do not precipitate. A young agar culture should be used, and a small quantity of the growth removed with the platinum needle and emulsified with distilled water in a clean watch glass. A drop of this emulsion is carefully spread over the surface of the clean coverslip and allowed to dry, and then passed once or twice through the flame. Three methods are described, although many others have been suggested from time to time. It will be seen that a mordant is used in all cases.

Pittfield's Flagella Stain.

Solutions:	A.	Saturated alcoholic gentian-violet ..	1 part.
		10 per cent. solution of potash alum..	10 parts.
	B.	10 per cent. solution of tannic acid	

Mix equal parts of A and B immediately before use.

Method: Flood the coverslip with the mixture and warm over bunsen flame till steam is given off, but do not boil. Allow the stain to remain on for five minutes; wash off, dry, and mount.

van Ermengem's Flagella Stain.

A.	Osmic acid 2 per cent. solution	1 ccm.
	Tannin 20 per cent. solution	2 ccm.
	Acetic acid (glacial)	5 drops.
	Mix, and keep till distinct violet colour.	
B.	Silver nitrate	0·5 gm.
	Distilled water	100 cc.
C.	Gallic acid	5 gm.
	Tannin	3 gm.
	Fused potassium acetate	10 gm.
	Distilled water..	350 cc.

Method: Place films in solution A for half an hour, wash well in distilled water, and then in absolute alcohol. Transfer to solution B for twenty seconds, and then without washing place in solution C for ten seconds. The film is again placed in solution B till it commences to turn black, and then washed, dried, and mounted.

McCrorie's Flagella Stain.

Solutions:	A. Saturated alcoholic "night-blue" ..	1 part.
	B. 10 per cent. solution potash alum ..	1 part.
	C. 10 per cent. solution of tannin ..	1 part.

Method: Mix immediately before staining, place film in incubator for half an hour. Wash, dry, and mount. The stain works best about an hour after mixing.

Neisser's Stain for Diphtheria.

A. Methylene blue	1 gm.
Absolute alcohol	20 cc.
Glacial acetic acid	50 cc.
Distilled water	930 cc.
Filter.	
B. Bismarck Brown	2 gm.
Distilled water	1,000 cc.
Filter.	

Stain one minute in A, wash, and stain one minute in B.

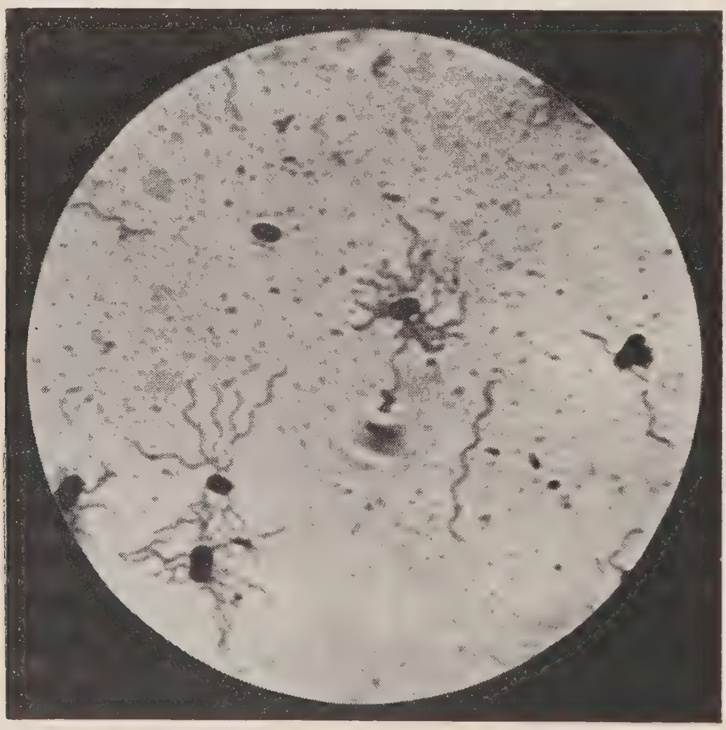

FIG. 17.—BACILLUS TYPHI ABDOMINALIS FLAGELLA STAINED BY PITFIELD'S PROCESS.
x 1,000.
From Washbourn and Goodall's Infectious Diseases.

CHAPTER V.

Methods of Observing Bacteria—Cultivations—Culture Media.

Methods of Observing Bacteria—Cultural.

In the cultivation or growth of bacteria in the laboratory various solutions and jellies are made use of, some of which are adapted to the development of the majority of micro-organisms, others adapted to certain species. These culture media contain the substances peculiarly suited to the growth of bacteria, while at the same time they are easy to manipulate.

The largest number of bacteria which are to be found in the mouth will grow perfectly well upon the commonly used laboratory media; notwithstanding this a considerable number of observers have adopted various special varieties of media with which to carry on their labours, without at the same time giving details of the growth upon the media common to all proper bacteriological work. In this way it is often most difficult to determine the exact relationship of many bacteria which have been described, often most imperfectly, as occurring in the mouth. Certain mouth bacteria no doubt require special media for their isolation, but once isolated they will generally develop on the usual laboratory test media. It is clearly essential therefore that the same methods and composition of media should be adopted in all descriptive work of new bacteria in order that real progress may be made; bacteria as a rule are so influenced by their environment that the greatest care has to be exercised in making the standard media.

I shall therefore give the details of ordinary laboratory media making in full, as adopted in the majority of bacteriological laboratories both in this country and on the Continent. The formulæ for special media are also given, the detail where omitted is similar to that described for the preparation of ordinary media. The

reader will have already gathered from the chapter on morphology that many species of bacteria have the same morphological form as determined by microscopical examination, and therefore the method of cultivation is invoked to establish easily recognised differences between the various species.

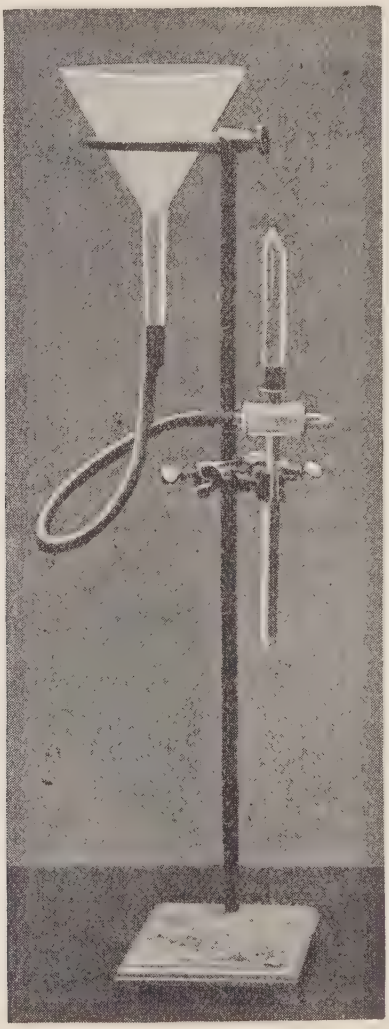

FIG. 18.—APPARATUS ARRANGED FOR FILLING TEST TUBES WITH NUTRIENT SOLUTIONS.

The glass three-way tap is connected to the reservoir funnel with a rubber tube. The liquid is allowed to flow from the funnel into the glass measuring cylinder at the top of the three-way tap till it reaches the 10 cc. mark; the tap is then turned sharply and the fluid flows into the test tube.

Culture media may be either fluid or solid, natural or artificial, the majority having a common basis of watery extract of meat; this will be described first.

Nutrient Broth or bouillon forms one of the most useful media, and moreover the basis of many of the jellies or solid media used.

Preparation.—A pound of lean beefsteak is finely chopped up and passed through a mincing machine (the fat and connective tissue are removed first). A litre of distilled water is added and the whole digested for half an hour at 60° C.; the mixture should be kept constantly stirred.

The temperature is now allowed to rise to 100° C. and the fluid filtered off through filter paper, and the filtrate made up to the litre with more water. To the filtrate 1 per cent. of peptone (10 gm.) and 0·5 per cent. (5 gm.) of sodium chloride is added. The peptone and salt are best mixed up with 25 cc. of the broth into a thick emulsion and then added to the bulk, boiled for half an hour, neutralised and filtered. The filtrate is *nutrient broth.* In preparing agar and gelatin, the agar or gelatin is added at the same time as the peptone.

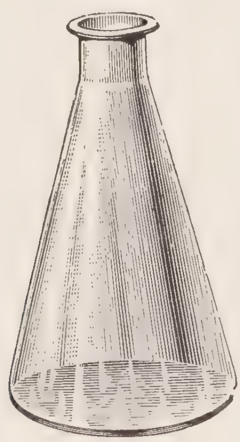

FIG. 19.—ERLENMEYER FLASK.

In neutralizing the broth two methods may be adopted, the point aimed at however should be to use a method which shall give constant results so that the finished media shall be of a definite alkalinity or acidity expressed in terms of standard alkali, otherwise successive brews will not have a constant definite reaction. Bacteria, as has been pointed out, are extremely sensitive to their environment, and the differences of alkalinity in two batches of media modify the cultural characters of many bacteria to a surprising extent. Considerable care must be exercised therefore in neutralizing media.

Phenolphthalein Method.—Reagents, &c., 5 per cent. of phenol-

phthalein in 50 per cent. alcohol. Burette graduated in tenths of a cubic centimetre. 25 ccm. pipette. Boiling distilled water in a wash bottle. Normal and decinormal sodium hydrate. Beakers.

Method.—Fill up burette with $\frac{N}{10}$ NaOH (10 cc. = 1 cc. $\frac{N}{1}$ NaOH). Withdraw 25 cc. of the broth (agar or gelatin) and run into the beaker, wash out the pipette with boiling distilled water into beaker, keep contents at boiling point. Add 3 or 4 drops of the phenolphthalein solution.

Read burette and carefully run in the $\frac{N}{10}$ NaOH until a faint flesh colour appears. Read burette. Make a control and adopt the mean.

The titration must be made at boiling to eliminate CO_2, which will interfere with the reaction.

The number of cubic centimetres of decinormal sodium hydrate used ($\frac{N}{10}$ NaOH) will give the number of cc. required to render 25 cc. of broth neutral to phenolphthalein.

Example—

Suppose that 25 cc. required 6·0 cc. of $\frac{N}{10}$ NaOH to produce a faint pink colour.
25 cc. ,, 6·25 cc.
25 cc. ,, 6·05 cc.

Average for three estimations 6·1 cc. $\frac{N}{10}$ NaOH.

Therefore—

100 cc. will require $6{\cdot}1 \times 4$,
And 1,000 cc. ,, ,, $6{\cdot}1 \times 4 \times 10 = 244$ cc. $\frac{N}{10}$ NaOH = 24·4 cc. $\frac{N}{1}$ NaOH.

But we have removed 75 cc. for estimation, therefore we have left $1000 - 75 = 925$ cc. It follows therefore that 925 cc. will require 22·6 cc. $\frac{N}{1}$ NaOH to render it neutral to phenolphthalein.

Only a few organisms will show maximum development in a medium with such a reaction, but it has been found by various workers, especially Eyre, that the reaction for the majority of bacteria is at an optimum when the medium still requires 10 cc. $\frac{N}{1}$ NaOH to be added per litre to bring the reaction up to neutral to phenolphthalein. The medium which still requires this 10 cc. $\frac{N}{1}$ NaOH per litre is said to have a reaction of "plus 10," or 10 degrees of acidity to phenolphthalein.

In the example above, then, it is necessary to deduct 9·25 from the estimated quantity for absolute neutralization (22·5 cc.), and the addition therefore of 12·4 cc. $\frac{N}{1}$ NaOH to our broth will give it a reaction of + 10. Other degrees are expressed as indicated + 5, + 4, + 8, and so on.

By this means it is always possible to prepare a medium of "standard reaction."

Litmus Method.—The hot medium (broth, agar, or gelatin) is gradually neutralized by dropping in $\frac{N}{1}$ NaOH from a burette and testing the reaction on litmus paper. When the medium is neutral to litmus 4·5 cc. more of $\frac{N}{1}$ NaOH are added to each litre. This method is much quicker, but does not take into account the NaH_2PO_4, and is therefore uncertain, especially as many weak organic acids do not react to litmus at all well.

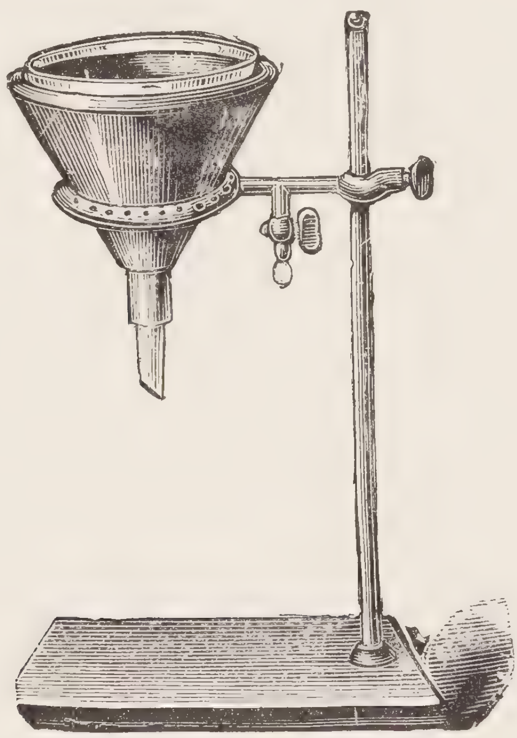

FIG. 20.—HOT-WATER FUNNEL.

Note.—In making up the filtrate to the litre when hot the method adopted is to weigh the medium in a tared flask and add the requisite amount by weight, having weighed the whole at the commencement.

(*a*) *Agar* (ordinary nutrient).—Powdered agar-agar, 2 gms.; nutrient broth, 100 cc.

The agar powder is mixed to a thin cream with some of the broth and added to the bulk. The mixture is then boiled in the steam sterilizer until all the agar is dissolved, neutralized while hot in the manner described for broth, and cooled to 60° C. The white of two eggs beaten up in distilled water are added, and the material boiled in the steamer until the whole of the flocculent precipitate

has fallen, which generally takes three-quarters to one and a quarter hours.

It is now ready for filtering; the ordinary filter papers are too fine for the purpose, and the ones generally used are the thick white "Chardin" filters. The filtering may be carried out in a hot water funnel (fig. 20), or in the steam sterilizer, and is a somewhat tedious process. When filtered the clear filtrate is passed into test tubes and sterilized by the intermittent method.

To avoid filtering the medium may be boiled, after the egg is added, in tall beakers and allowed to cool therein; the flocculent precipitate falls to the bottom and may be removed with a knife when the mass has solidified. The agar made in this way is not so clear as when filtered.

Some bacteriologists boil their agar in the autoclave, but the great objection to this is that the medium tends to turn brown.

(*b*) *Glycerine Agar.*—This medium, first introduced for the culture of the tubercle bacillus by Roux and Yersin, is ordinary nutrient agar to which 10 per cent. of glycerine has been added. The preparation is as (*a*).

(*c*) *Glucose Formate Agar.*—This medium, which is largely used in the cultivation of anäerobic organisms, is ordinary nutrient agar to which 2 per cent. of glucose and 0·5 per cent. of sodium formate have been added. Kitasatio, who introduced the method, did so on purely theoretical grounds, the elaboration of proteid taking place in its primary synthesis by the formation of aldehyde (Hueppe).

(*d*) *Agar Streaked with Blood.*—This medium is used in the cultivation of the pneumococcus, and gonococcus particularly. Fresh human blood may be used, or that of an animal; in either case great care must be exercised in excluding adventitious bacteria. The method generally adopted is to take a rabbit, wash the ear well with lysol and soft soap and shave off the hair. Again wash with lysol and finally with alcohol. The large vein is then punctured, and the escaping blood removed with sterile pipettes and smeared over the surface of slanted agar tubes. The tubes prepared in this way are incubated for twenty-four to forty-eight hours in the hot incubator at 37·5° C., and if no development of colonies takes place are ready for use.

Various other substances are added to agar for special purposes; the basis in all cases is the ordinary nutrient agar (*a*). The following are some of the varieties:—

(e) *Iron Agar.*—2 per cent. of saccharate or tartrate of iron.

(f) *Sugar Agar.*—Maltose and lactose, 5 per cent., &c.

(g) *Gelatin Agar.*—Broth, 100 cc.; agar, 1·5 gm.; gelatin, 7·0 gm. Prepared as agar (a).

(h) *Gelatin.*—Ordinary nutrient gelatin is a medium largely employed. Its composition is: best French gelatin, 10 gm.; nutrient broth, 100 cc.

The gelatin is dissolved in the broth by heat, neutralized while hot (as above), cooled to 60° C., the white of an egg added, boiled for half an hour, filtered in a hot water funnel and run into sterile tubes, and sterilized by intermittent method.

The sterilization of gelatin in the autoclave generally results in the peptonisation of the gelatin, in which condition it will not set on cooling; 20 per cent. gelatin is also employed at times.

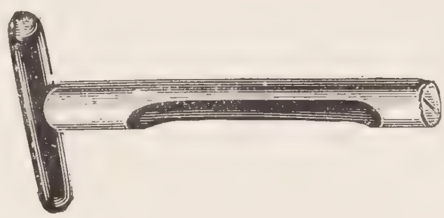

Fig. 21.—Potato Cutter.

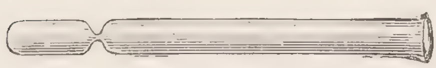

Fig. 22.—Roux's Potato Tube, the lower end arranged to catch condensation water.

(i) *Glycerine Gelatin.*—Gelatin, 10 gm.; glycerine, 4 ccm.; broth, 100 ccm. Prepare as gelatin.

(j) Glycerine may also be added to ordinary broth in the same proportion (4 per cent.).

(k) *Potato.*—A good sized potato is well washed with a brush and hot water, peeled, and the eyes removed. With a circular potato cutter (fig. 21) cut out cylinders about four inches long, and wash well with water. Divide the cylinders longitudinally so that each has a broad and narrow end. Drop them into sterile Roux's tubes or tubes with small plugs of wool at the bottom and sterilize in the steamer. If the potatoes are acid wash the cut slices in 2 per cent. caustic soda for an hour before placing in tubes. Glycerinated potato is also used. The slices are soaked in 6 per cent. solution of glycerine in water before they are placed in the tubes.

(*l*) *Potato Gelatin.*—Peel several potatoes and remove the eyes, weigh out 1 kilo., cut up in mincing machine, and add 1,000 cc. of water. Allow to stand twenty-four hours. Filter. Add 1 per cent. asparagin and 4 per cent. glycerine, 10 per cent. gelatin, and the white of an egg. Boil up, filter, and run into tubes.

Potato water is made in a similar way, but without gelatin.

(*m*) *Neutral Litmus.*—Two ounces of commercial litmus are extracted with rectified spirit for thirty days, changing the spirit three times. At the end of this time the litmus is emptied into a flask, and the spirit allowed to evaporate ; 600 cc. of filtered water are next added, the litmus dissolved up and filtered, and acidified with pure sulphuric acid. An excess of barium hydrate is then added, and the solution again filtered. Carbon dioxide is then passed through till all the barium is precipitated as carbonate, the solution filtered and sterilized.

Sufficient of this solution to give a good blue colour is added to the various media when required.

(*n*) *Litmus Milk.*—500 cc. of milk are run into a funnel and heated in the sterilizer, and allowed to stand for twenty-four hours till the cream has risen. The milk is then drawn off, tinted by addition of neutral litmus, run into tubes and sterilized in the steamer.

(*o*) *Blood Serum.*—The blood of an animal is collected in a large sterilized jar at the slaughter house ; the first runnings are allowed to escape to avoid contamination from the skin, &c. The jar is then filled and the blood allowed to clot. The serum is pipetted off with a sterile pipette and run into tubes, which are placed in the inspissator in a slanting position, and sterilized by heating to a temperature of 75° C. for half an hour on four or five successive days. Reject any tubes that show growth when incubated after the last sterilization.

(*p*) *Peptone Water* (Durham's).—Water 100 cc. ; peptone 1 gm. ; salt 0·5 gm. Boil for twenty minutes, filter and run into tubes and sterilize in the usual manner. Instead of 1 per cent. more peptone may be added up to 4 per cent.

Peptone water is a useful medium to use in determining the fermentation of carbohydrates. Various amounts may be added, 2 per cent. being an average quantity. The solution may be also coloured with neutral litmus. Glucose, lactose, maltose, starch, &c., may be used.

(*q*) *Beer Wort Gelatin, and Agar.*—Beer wort is used instead of broth in making these media; it is best not to neutralize.

(*r*) *Nitrate Media.*—0·5 per cent. of potassium or sodium nitrate may be added to test the reducing power of organisms upon nitrates. Broth, gelatin or agar may be used.

(*s*) *Bread* is a good medium for moulds. Dry bread is grated and the crumbs placed in small Erlenmeyer flasks and just covered with distilled water. The flasks are sterilized on four succeeding days in the steamer.

(*t*) *Media with Iron Salts.*—0·2 per cent. of iron lactate or saccharate may be added to agar, gelatin or broth, for testing the production of sulphuretted hydrogen.

(*u*) *Inosit Free Broth.*—Bacillus coli is grown in the beef extract before addition of peptone, &c. After eighteen hours at 37° C. the flask is placed in the steamer for an hour and the contents subsequently used for making agar, gelatin or broth. The organisms use up the muscle sugar (inosit).

(*v*) *Saliva Media.*—Saliva is obtained by placing a sterile Woolfe bottle on the draining tube of a saliva ejector whilst the latter is in use for dental operations. The saliva is boiled, 1 per cent. peptone added, filtered and sterilized in the tubes. Gelatin 10 per cent. or agar 2 per cent. may be added for solid media. The saliva should be sterilized as soon after collection as possible; if not treated in four hours it must be rejected. For some operations the fresh saliva is filtered through a Pasteur-Chamberland filter. (Figs. 11 and 30.)

Methods of Cultivating Bacteria.

Inoculation of Culture Tubes.—The operation of planting a substance containing organisms on to nutrient media for the purpose of obtaining a culture is termed inoculation, and is performed with a platinum wire mounted in a glass, or better, aluminium handle. Two wires are required: (*a*) a straight wire slightly flattened at the end into a spatula; (*b*) a wire terminating in a loop—two of these, different sizes, are useful (fig. 23). Before and after use the wire is heated to redness in the flame, before use to burn off any adhering organisms that would contaminate the culture, afterwards to remove the organisms taken from the tube and still remaining attached to the wire.

To inoculate one tube from another containing a cultivation

the following method is adopted :—Flame, *i.e.*, set fire to the cotton wool plugs of the two tubes to burn off any dust (bacteria) which may have fallen on to the stopper, then blow out the flame. Hold the tubes together in the left hand between the thumb and first two fingers, take up the platinum wire like a pencil and sterilize it in the flame; with the little finger of the right hand remove the cotton

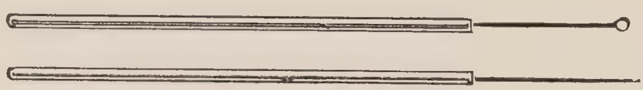

Fig. 23.—Platino-iridium Inoculating Needles, Loop and Spatula.

wool plug from one of the two tubes, keeping them inclined at an angle to avoid spores, &c., dropping in; remove the second plug with the third finger, holding the two plugs in the right hand (see fig. 24). Flame the open mouths of the two tubes and then remove a small portion of the culture from one tube and transfer it to the other,

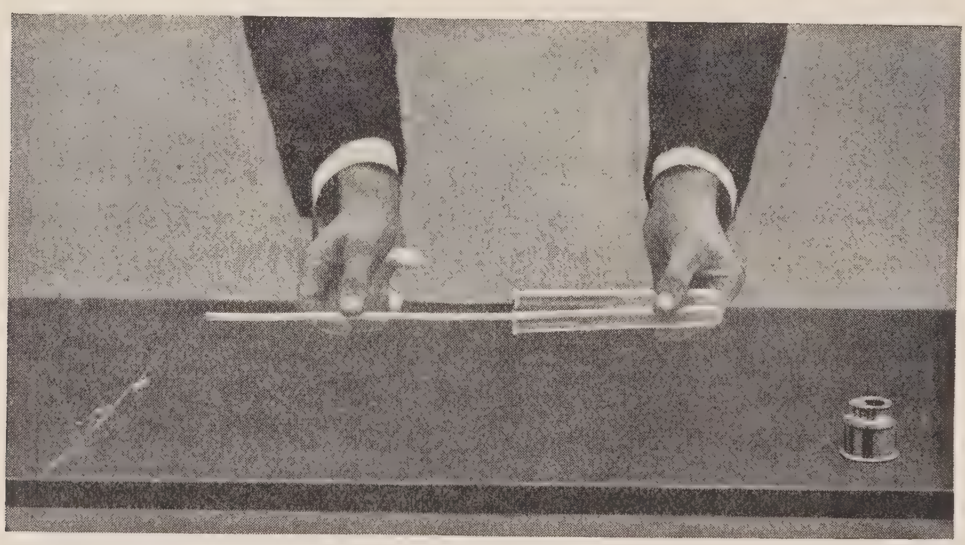

Fig. 24.—Method of Inoculating one Tube from Another (viewed from above). Note the method of holding the cotton-wool plugs.

carefully avoiding touching the edge or sides of the tubes in the process; replace the plugs, flame the wire, and then the tube plugs again, label and place the inoculated tubes in the incubator. Exactly the same procedure is adopted if the cultivation is made from any material from which we wish to cultivate bacteria; when the material is fluid the loop is used.

Solid media are inoculated in three ways: (*a*) "streak," (*b*) "stab," (*c*) "shake."

Streak Cultures.—The media is first "sloped," *i.e.*, melted, and the tube laid down at an angle, care being taken that the fluid does not touch the plug; several tubes should be sloped at once, and a folded duster placed over them while they cool, to prevent dimming

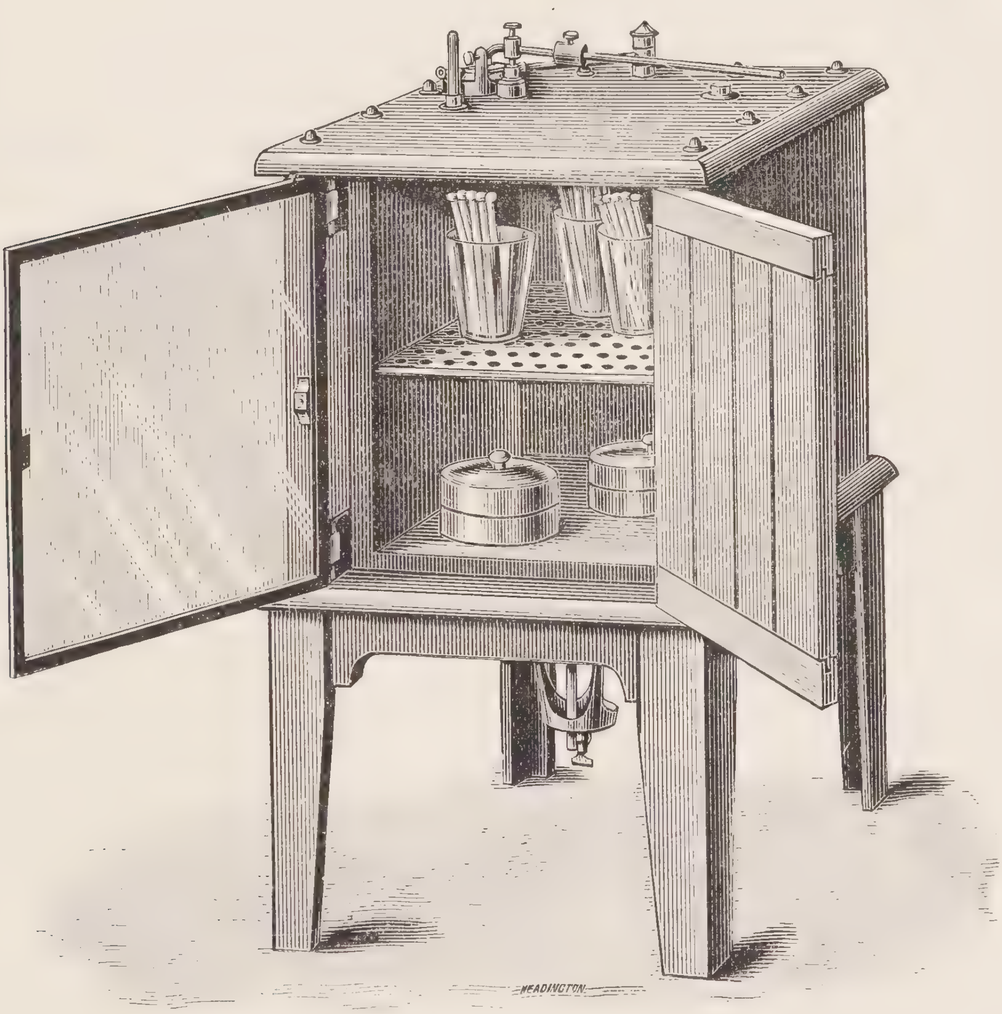

FIG. 25.—HEARSON'S INCUBATOR.

The apparatus consists of a copper tank filled with water enclosed in a wooden frame. The temperature is maintained by a gas jet, the supply to which is regulated by a capsule with a definite boiling point. The excursions of the capsule are communicated to a valve which automatically regulates the gas supply. Two incubators are required, one working at 37·5° C., the other at 22° C.

by condensation. When sloped the tubes should be kept twenty-four hours before using. With agar it is a good plan to add a little gum arabic or gelatin to the media to prevent the sloped media slipping to the bottom of the tube. To inoculate the sloped surface

draw the platinum needle or loop, charged with the culture, gently up the surface of the medium and replace the plug. The condensation water in agar tubes should not be poured out, as it has a characteristic appearance with certain organisms.

Stab Cultures.—These, like the latter, may be made on agar or gelatin; the tubes are not sloped. A charged needle is passed to the bottom of the medium and withdrawn. This method is largely used in making cultures of anäerobic bacteria and determining the production of liquefaction in gelatin.

Shake Cultures may be used to determine anäerobiosis, production of gas, &c. The solid medium is melted in a water bath, conveniently fitted with a rack perforated to allow of the tubes standing upright; when *thoroughly* melted the medium is allowed to cool to 40° C., inoculated as described above, and then well shaken by four or five rapid swings (not up and down). When set the tube is placed in the incubator.

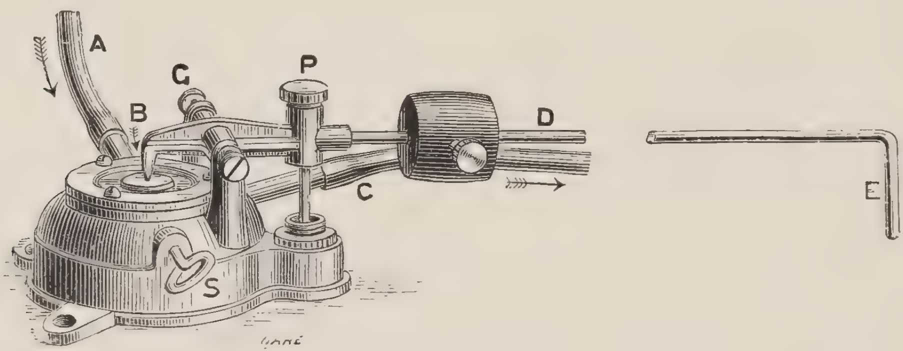

FIG. 26.—ENLARGED VIEW OF GAS VALVE IN HEARSON'S BIOLOGICAL INCUBATOR.

A, Gas supply; B, tambour; C, gas supply to jet; D, weight on lever to regulate size of flame; P, pin communicating with capsule in the incubator; S, sideway tap.

Liquid Media.—The loop or spatula charged with material should not be directly inoculated into the fluid, but the inside of the tube just above the meniscus is touched with the charged wire; a loopful of the medium is then taken up and mixed with the drop of material so that a complete emulsion is formed. On placing the tube upright and giving it one or two swings the material is diffused through the tube.

Plate Cultivations.—Bacteria rarely exist in nature alone and are invariably associated with other species, it is therefore necessary for the bacteriologist to adopt a method of separating the various

species. This is usually done by means of plates The procedure is the same for gelatin and agar.

Three tubes of agar or gelatin are melted in the water bath and cooled to 40° C., and one tube inoculated with the mixed culture and shaken up. Two loopfuls of this tube are now transferred to a second tube and four or five loops of the second into the third. The plug of each tube is removed and the contents poured into a sterile Petri dish, which has been placed upon the levelling tripod, the glass dish of which has been filled with warm water at 40° C. The mouth of the tubes are flamed before pouring out the contents, and the lip of the tube used to assist the medium to flow over the dish. After the plates have set they are incubated. In summer it is often necessary to put ice into the reservoir water, otherwise the gelatin does not set for hours.

The amount of media placed in the first tube determines the number of loopfuls used for the subsequent dilutions, in fact the whole process can only be satisfactorily learned by practice in the laboratory.

In determining the number of bacteria present in a sample of, say, drinking water, a sterile pipette graduated in tenths of a cubic centimetre is used, and 0·5, 0·3 and 0·1 ccm. of the water added to the various tubes, which are then plated.

In the process of plating the point aimed at is to separate the bacteria from one another in such a manner that when they develop in the nutrient substratum the colonies each one forms are sufficiently separated to observe and make sub-cultivations from, or in the water-dilution plates to count the individual colonies, each colony representing one organism in the original sample, the total number of colonies on the three plates representing the number of bacteria present in a cubic centimetre of the water examined. A sub-culture made from one of these colonies will generally be found to be pure, *i.e.*, will consist of one species of organism alone.

Having obtained cultivations from a plate colony the culture is examined by means of the hanging drop and coverslip preparation stained in the various methods given above, and then sub-cultured into the various test media, coverslip preparations of each being made.

The test media to be used should always include the following:—gelatin stab, streak, shake, plates, agar streak, broth, litmus milk, blood serum, potato, media containing carbo-hydrate, and others containing nitrate.

So far I have only described the cultivations of äerobic organisms, the anäerobic bacteria requiring special methods.

Glucose formate media are especially adapted to the growth of anäerobes, but other media may be used.

The oxygen of the air must be excluded by one of the following methods:—

(*a*) *Buchner's Tubes* (fig. 27).—A large boiling tube fitted with an india-rubber cork is used, and a little pyrogallic acid placed in the

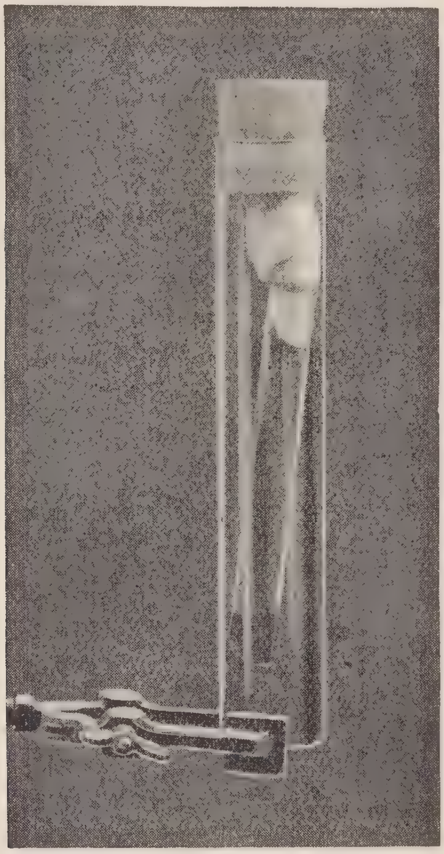

Fig. 27.—Buchner's Tube for Anäerobic Cultivations, with Culture Tube in Situ.

bottom; some caustic soda solution is then added, the culture tube, previously inoculated, gently dropped in and the rubber cork replaced. This method is adapted for fluid or solid media.

(*b*) An agar or gelatin stab is made in a tube containing about twice as much of the medium as is used for ordinary purposes, and the top of the stab covered by pouring in a little agar or gelatin which has been melted in another tube.

(*c*) Hydrogen or other indifferent gas (p. 17) is used to drive out

the air. The culture may be placed in a special jar with two tubes leading into it (fig. 28). Along one is passed hydrogen washed by passage through (*a*) 10 per cent. solution of lead acetate, (*b*) 10 per cent. solution of silver nitrate, (*c*) 10 per cent. solution of pyrogallic

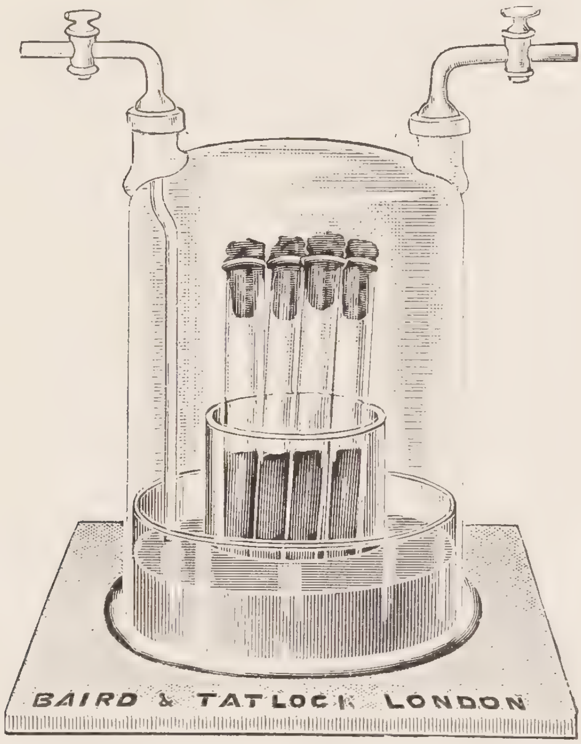

FIG. 28.—BULLOCK'S ANÄEROBIC APPARATUS FOR HYDROGEN AND ALKALINE PYROGALLIC ACID.

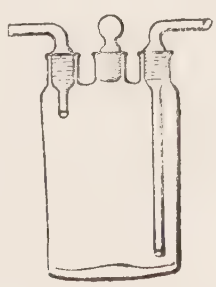

FIG. 29.—WOLFF BOTTLE FOR USE WITH BULLOCK'S APPARATUS.
Three are used: (*a*) lead acetate solution; (*b*) silver nitrate solution; (*c*) pyrogallic acid and soda.

acid in caustic soda. Both inlet and outlet tubes are provided with cocks which are closed when the gas has been passed till no air remains. The lead solution removes any H_2S, the silver solution any Cl, and the pyrogallic acid traces of oxygen. The air should also be filtered through cotton wool to remove any bacteria.

Sloped tubes with a tightly-fitting indiarubber cork may be

used in the same way, the two tubes being sealed in the flame when the gas has been passed for nine or ten minutes.

Vignal's Method is often a useful one. A piece of thin glass tube is heated till it is sterile and either end drawn out. The inoculated and melted medium, glucose formate agar for instance, is then drawn up into the tube until it is quite full, when the ends are sealed by fusing in the flame. It is best to pass hydrogen through the tube beforehand. To obtain sub-cultures the tube is marked with a file opposite a colony and the tube broken across.

Anäerobic cultures in fluid media are also made by using an ordinary filter flask and passing hydrogen over in the manner described above.

If, as is often the case, we wish to collect and examine the gas produced by anäerobes, a flask, a bent tube—the end of which dips into a trough of mercury—is used and the gas collected by displacement.

The Study of Cultures.

In order to properly recognise a given organism it is necessary to make use of the culture media whose composition and mode of preparation has been considered.[1] Some organisms, it is true, have typical and well-marked characters upon certain media, and an experienced eye can therefore pick out some of the well-known forms; but for all general purposes of recognition and differentiation the appearances and chemical reactions on a number of different media must be employed (for chart see page 69). The study of cultivations, therefore, gives the mycologist his methods of detecting and isolating any particular bacterium, and it is therefore of the greatest importance that some general scheme be adopted, both for the convenience of the individual worker and for the interests of the science at large.

The descriptions of cultural characters given by many workers are often unintentionally misleading from the unavoidable fact that many organisms do not conform to a rigid rule, and moreover from the avoidable confusion of not using standard media.

To facilitate the general description of bacteria, Chester has recently suggested the introduction of the general botanical nomenclature in the description of cultural characteristics, the terms used indicating general types rather than minute and particular descrip-

---

[1] See page 51.

tions of isolated examples. Where possible I have placed these terms in brackets; the descriptive terms suggested by Chester, with diagrams of the various forms to which the terms apply, are given in the appendix.

The colonies formed by bacteria upon plates of nutrient media should be carefully examined both with the naked eye and with a $\frac{2}{3}$ objective, many bacteria producing typical forms on the various media. Both deep and surface colonies should be examined, the colonies in the deep layers often appearing to differ widely from those growing on the surface, even in pure cultivations.

On the sloped surface of agar, gelatin, blood serum, potato, or other solid medium the general characters of the growth, the contour of the edge, the surface, its consistency, &c., are to be noted, as well as changes in the medium, such as liquefaction and pigmentation.

When a pigment is formed the solubility should be tested in various solvents, and, if possible, the spectrum noted. In stab cultures several other points are generally noted—the special form in which liquefaction of gelatin occurs, the formation of gas and the presence of growth along the track of the inoculation needle, the character of the surface of the growth at the point of entry of the needle, &c. (See diagrams in appendix.)

In fluid media, besides the general character of the growth, note pellicle, or precipitate, turbidity, gas production and acid production, as by the alteration in the tint of litmus added to the solution various chemical tests may also be applied.

In broth cultures various other tests may be applied for ammonia, nitrates, sulphuretted hydrogen, &c.

Peptone water, or broth cultures, with the addition of 1 to 2 per cent. of various carbo-hydrates, are used for the determination of fermentation indol and acid production. For gas formation Durham's tube is most useful. A small test tube is placed in the culture and sterilized with the medium; any gas formed collects in the floating tube, displacing the contained fluid.

Theobald Smith's tube may be also used. The bulb is filled with glucose-broth and the gas formed collects in the large end of the tube, from which it may be removed for analysis. The amount of CO_2 may be determined by filling the bulb with NaOH and shaking up; the loss in volume represents the CO_2 absorbed.

Proteolytic enzymes may be also tested for in broth cultures as follows:—A broth culture of known liquefying organism is

grown for four or five days, and at the end of this time some of the contents are poured into a tube of thymol gelatin and a small piece of thymol added. A control tube is filled with water and thymol added. If any enzyme is present liquefaction takes place in the tube containing the culture, the organisms being prevented from developing by the thymol.

The enzyme may be isolated if desired by extracting with thymol water, precipitating with absolute alcohol, and taking up precipitate in thymol water again. Certain sugars which do not reduce Fehling's solution are inverted by bacterial activity into reducing sugars; these are tested for with Fehling in the ordinary way.

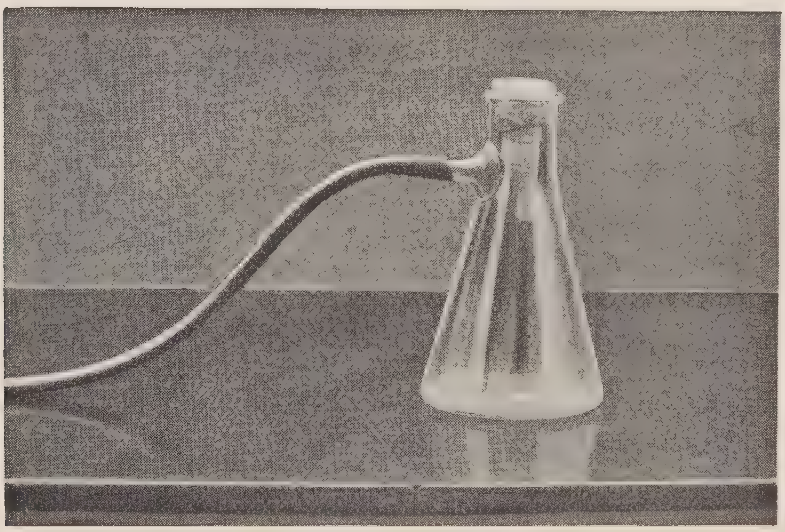

Fig. 30.—Filter Flask with Pasteur-Chamberland Filter ready for Filtering Toxine. The rubber tube is connected with an exhaust pump.

Alkali albumin in broth and ordinary nutrient broth are used to obtain the toxins of certain bacteria, such as diphtheria. The organisms are grown for seven days at 37° C., and then filtered off by means of a porcelain filter candle (fig. 30); the filtrate containing the toxins may be tested by injection into animals.

For the isolation and quantitative determination of the various organic acids, &c., produced by bacteria from carbo-hydrates, fractional distillation must be employed, details of which are given in appendix.

CHART FOR STUDY OF BACTERIA.

Impure Cultivation plated—Colony picked out and planted on to Agar, Broth or Gelatin.

Pure Cultivation.

A	B	C
Microscopical Examination of Stained Coverslip preparations	Microscopical Examination of Hanging drop	Cultivation Experiments
Gram's method	Motility	Agar (Solid media)
Carbol methylene blue	Spores	Gelatin (Solid media)
Fuchsin	Spore germination	Blood Serum (Solid media)
Flagella staining	Chemiotaxis	Potato (Solid media)
Spore staining	Agglutination	Broth (Liquid media)
Ziehl-Neelsen	Bacteriolysis	Milk (Liquid media)
Capsules	Plasmolysis	Carbo hydrates (Liquid media)
Plasmolysis		Peptone water (Liquid media)
		Nitrate (Special media)
		Iron, &c. (Special media)

General Characters of Cultivations at 37·5° and 22° C.

Gelatin (22° C)	Agar	Blood Serum	Potato	Broth	Milk	Carbo-hydrates	Peptone Water
Plates—colonies	Streak	Streak	Streak	Pellicle	Coagulation	Acid	Indol and other
Streak	Stab	(liquefaction, &c.)	(Pigment)	Precipitate	Acid	Gas	Aromatics
Stab—liquefaction				Chemical reaction	Smell		Ammonia
Shake—gas, liquefaction, &c.				(Indol, &c.)			

Stained preparations of twenty-four and forty-eight hours' growth on all media. Reactions on other media (special).
Thermal death point. Spore resistance. Optimum reaction, &c.
Pathogenesis.

Pathogenesis.

Notwithstanding the statements of a certain small class of unscientific persons the experimental inoculation of animals has undoubtedly led to great advances in medicine and surgery redounding with benefit to the human race, and has placed many new and valuable facts of treatment and diagnosis within the reach of those engaged in alleviating suffering and combating disease.

The animals chiefly used in experimental inoculation by bacteriologists licensed to perform such experiments are rabbits, guinea pigs, rats and mice, whilst in the production of antitoxine on a large scale horses are generally employed. The experimental inoculation may be made in various ways.

(*a*) *Subcutaneous Injection*, the material used being injected under the skin on the abdominal surface in guinea pigs and rabbits, and at the root of the tail in mice. Solid matter is thus introduced.

(*b*) *Intra-peritoneal Inoculation.*—The fluid is introduced into the peritoneal cavity direct by means of a sterilised hypodermic syringe.

(*c*) *Intra-venous Inoculation*, the injection being made directly into the veins, generally the posterior vein of the rabbit's ear.

(*d*) *Intra-muscular Inoculation.*—The injection is made deeply into the muscles. Various other methods are also employed, such as mixing the infecting material with the animal's food, intercranial injection by removing a portion of the skull with a small trephine; inoculation into the anterior chamber of the eye, &c. The animal is placed in a separate cage after inoculation and watched, the symptoms noted, and if death occurs a careful autopsy made. To avoid any contamination sterile instruments (well boiled) are used, and the surfaces of glands, heart, &c., well seared with a hot iron before cultivations are made. Cultivations and coverslip preparations are made from the site of inoculation, peritoneal fluid, heart blood and spleen, and from any other sites that the special case under consideration suggests.

For the full particulars of experimental inoculation the reader is referred to the laboratory text-book by Eyre;[1] and it must be remembered that, owing to the stringent regulations in force, no one may perform inoculation experiments under pain of heavy penalty unless they hold a licence from the Home Secretary.

---

[1] "Bacterological Technique."

CHAPTER VI.

Susceptibility and Immunity.

Immunity.

General.—By immunity is meant the non-susceptibility to a given disease or to a given micro-organism. Such a resistance may be natural to a whole genus of animals or may be acquired by an animal as the outcome of experimental inoculation; immunity is therefore divided into two great divisions—*natural immunity* as possessed by certain animals naturally, and *acquired immunity* developed either as the result of passing through an attack of the disease, or as the result of experimental inoculation with the agents of the disease (bacteria or their toxines). All diseases do not produce a corresponding immunity, whilst others apparently confer a very large degree of protection. For instance in the case of small-pox, scarlet fever and typhoid fever (enteric), one attack generally produces protection lasting many years. On the other hand influenza, pneumonia and erysipelas may occur several times in the same individual, but even with these a certain transitory degree of immunity may be produced. All individuals do not exhibit the same degree of susceptibility to a given disease, as is seen in the percentages of those developing a disease after exposure to infection (*cf.* diphtheria).

The immunity shown by various races of man differs for certain diseases, and many diseases affecting man are unknown in the lower animals and *vice versâ*.

As a general rule the greater the virulence of the organism producing a disease the more pronounced the protection on recovery; in the experimental production of immunity therefore organisms of a high degree of virulence are employed in the final inoculations.

Changes in the Animal organism associated with Immunity.—In acquired immunity, and to a small extent in natural immunity, the

blood of the immunized animal is found to contain certain bodies which were not present before the disease was contracted or the special inoculations commenced, and the development of immunity is coincident with their appearance in the blood. These anti-bodies (antikörper) may be of two varieties : (*a*) *anti-toxic*, which neutralize the toxines produced by the invading bacteria ; (*b*) *anti-bacterial*, which attack and produce solution of the bacteria themselves. These anti-bodies are not always produced in equal quantities and vary within wide limits. The antitoxines used therapeutically as in treatment of diphtheria, are "antitoxic" rather than "anti-bacterial." The formation of anti-bodies is not limited to the infection by bacteria by their products, for instance the injection of the washed red blood corpuscles of one animal into another results in the formation of "*hæmolysins*" which produce solution of the red corpuscles of the blood injected, and so on with various other substances. Rennet ferment on injection produces "anti-rennin," which prevents rennet ferment from acting upon milk. Besides the production of these anti-bodies "*agglutinins*" are formed which cause the agglomeration of the bacteria against which the animal has been immunised. These bodies are referred to in greater detail below.

Artificial Immunity.—Two varieties are recognised: (*a*) Active immunity ; (*b*) Passive immunity (protective).

Active immunity is obtained by injecting an animal with a non-fatal dose of a given bacterium, the dose being so arranged that a considerable illness (reaction) with recovery ensues. After an interval the injection is repeated with a slight increase in the dose, and so on until the animal can withstand many times the initial fatal dose (determined by the injection of a control animal of the same size and weight) without reaction. The animal is now said to be "immunized." Instead of the bacteria themselves, their products, obtained by filtering broth cultures through porcelain filters, may be used, the animal developing immunity as the result of the injection of the toxines in the filtrate.

Active immunity may be produced :—

(*A*) BY THE INJECTION OF LIVING ORGANISMS ATTENUATED IN VARIOUS WAYS.

(1) *By growing in the presence of air or oxygen.* All pathogenic organisms gradually lose their initial virulence when cultivated for some time outside the body.

(2) *By the inoculation of another species of animal.* Thus passing anthrax through a guinea-pig lessens its virulence for cattle.

(3) *By cultivating the organisms at abnormal temperatures.* Pasteur found that anthrax was so much attenuated that it no longer produced fatal illness in sheep, if the cultures were exposed for a certain time to a temperature of 55° C.

(4) *By growing the organisms in weak antiseptic solutions,* and sometimes by injecting such solutions with the organism.

(5) *By injection of non-fatal doses of virulent organisms.*

(*B*) BY THE INJECTION OF DEAD ORGANISMS.

(*C*) BY THE INJECTION OF FILTERED BACTERIAL CULTURES.

(*D*) BY FEEDING WITH DEAD CULTURES OF BACTERIA.

Sometimes it is necessary to increase the initial virulence of a given organism.

EXALTATION OF VIRULENCE.

(*A*) *By the method of "passage,"* first described by Pasteur.

An animal is injected with an organism either *intravenously* or *intraperitoneally*, and another animal injected with the blood of the first, containing the organisms; or a culture may be made upon each occasion. By this means the virulence of the organism may be raised to an enormously high pitch.

(*B*) *By the combined injection with other organisms.*

Thus an attenuated diphtheria bacillus may be raised in virulence by injecting it into an animal together with the streptococcus pyogenes; an attenuated streptococcus by adding bacillus coli, &c.

(*C*) *By artificially lowering an animal's resistance* by the action of heat, cold or overwork, &c., or by exposure to general depressing conditions, *i.e.*, guinea-pigs exposed to sewer air succumb to smaller doses of diphtheria than do control animals.

By keeping a frog at a temperature of 30° C. it is rendered susceptible to anthrax and by cooling the ordinary fowl by iced water it becomes less resistant to the cholera vibrio.

By neutralizing the stomach contents of an animal immune to cholera and paralyzing the peristaltic action of the intestines with opium Pasteur found choleraic symptoms were produced on feeding with the organisms.

Passive Immunity is that form of resistance to a given disease conferred on a susceptible animal by injecting it with the serum of an immune animal.

The immune serum may be inoculated with the cultivation to be tested, or subsequently within certain time limits which differ for the different organisms used.

For instance, an animal A is injected with increasing doses of diphtheria toxine until it becomes immune. The animal is now bled under aseptic precautions and the clear serum separated from the clot.

An animal B, which has not undergone the process of immunization is injected with a fatal dose (determined by previous injection of other animals) of the diphtheria toxine, and at the same time with the serum obtained from A, B is protected and recovers from what would prove a fatal issue without the protection afforded by the serum of A. Such serum is termed *antitoxic*, as it apparently neutralizes the toxine. If the animal A is immunized by the injection of bacteria instead of their *toxines* the serum will protect against living organisms, that is, the immune serum is *antibacterial.*

Passive Immunity may therefore be divided into varieties.

(*A*) Antitoxic—when the anti-serum neutralizes the toxine.

(*B*) Antibacterial—when the anti-serum destroys the bacteria themselves.

It follows, therefore, that an antitoxic serum is obtained from an animal immunized by toxine injections, and an antibacterial serum from an animal immunized by the injection of living bacteria. The injection of killed cultures also produces an antibacterial serum containing lysins. The serum of an animal immunized by injection of living bacteria which produce a toxine may be both antitoxic and antibacterial, the two phenomena depending on different reactions.

The production of antitoxine in immunized animals has been applied to the treatment of various diseases of a toxic nature, especially diphtheria. The antibacterial sera have so far not met with any great success in the treatment of disease, although attempts have been recently made to protect man against enteric

fever by direct inoculation of killed typhoid cultures. This method, however, does not come under the head of anti-bacterial serum, the protection being produced by *active immunization.* The anti-bacterial bodies are important in the diagnosis of certain diseases, and will be referred to subsequently.

Antitoxine. Antitoxic Sera.—The various steps in the production of antitoxine are as follows, and diphtheria may be taken to serve as the type of the general method adopted:—

(1) *Preparation of a powerful toxine* by growing the organism elaborating the toxine under the most favourable conditions for the development of the poison. Filtration of the fluid containing the toxine through a porcelain filter (see fig. 30), the filtrate being termed "toxine."

(2) *Estimation of the power of the toxine* by inoculation of guinea-pigs to determine the minimal dose which will produce death.

Behring denotes as a normal poison a toxine solution of which 0·01 cc. is sufficient to kill a guinea-pig weighing 250 gm. in four days. Of this normal toxine (D.T.N.) 1 cc. will kill one hundred guinea-pigs. This is the toxine unit and has a working value of 25,000,[1] a toxine ten times as strong is expressed thus D.T.N.^{10}; one ten times weaker $\frac{\text{D.T.N.}}{10}$

(3) *Production of an antitoxic serum* in a susceptible animal (horse) by repeated toxine injections as described above (p. 72).

(4) *Estimation of the antitoxic power of the serum* by determining how much is required to protect against the normal diphtheria toxine unit (D.T.N.). If, for instance, the serum is found to contain enough antitoxine in 1 cc. to prevent a fatal result with the injection of 1 cc. of the toxine (D.T.N.), it is called a normal diphtheria antitoxine (D.A.N.). *The amount of antitoxine that is required to protect* 25,000 *gm. weight of guinea pigs from the minimal fatal dose of the poison* (D.T.N.), is termed an IMMUNITY UNIT (I.E.).

To cure a person ill with diphtheria 600 to 1,800 I.E. are required contained in 2—6 cc. of serum; therefore the strength of the serum used is D.A.N. 300.

Ehrlich has propounded the idea that the antitoxines are exactly identical with the portions of the proteid molecule to which the toxines become bound in the production of disease; the poison-susceptible parts of the molecule (toxophoric) are termed "side

[1] That is, 25,000 grammes weight of living guinea-pig.

chains" (seitenketten). When a toxine is introduced in small quantities, some of the "side chains" unite with the toxine, are thrown off from the cell and at once replaced. This repair takes place more and more rapidly as the side chains are more frequently torn off by the toxine. Finally the side chains are produced in greater quantity than there is toxine to fix and they then appear in the serum as antitoxine.

The production of antitoxine is apparently associated with an increase in the white blood corpuscles.

For a summary of the present knowledge of antitoxine and the theories of immunity the reader is referred to the article by Prof. Ritchie, *Journal of Hygiene*, April, 1902, and to the summary by Armit in the *British Medical Journal*, April 2, 1902.

Antibacterial Sera.—In certain diseases the production of immunity is associated with the production of bodies in the serum which possess a destructive action upon the bacteria themselves. These bodies may be shown to be present in various ways :—

(1) *Agglutination* (Gruber and Durham, Widal).
(2) *Bacteriolysis :* Pfeiffer's reaction.
(3) *Inhibition of growth* (Wright).

Fig. 31.—Widal Blood Pipette for collecting Blood for Agglutination Reaction.

(1) *Agglutination.*—Two or three drops of blood of the immunised animal are collected in a tube having a bulb in the centre and capillary ends (fig. 31); the blood is allowed to clot and the serum collected in the capillary end opposite to that by which the blood has been introduced. A cultivation of the organism in broth is taken and a drop of the diluted serum added, the reaction being carried out in a small test tube. If the organism be, say, B. pyocyaneus and the animal was immunised to B. pyocyaneus, a well marked precipitate soon occurs. If instead of the test tube the reaction is carried out on a hanging drop slide and watched under the microscope the motile organisms will be seen to lose their motility and become aggregated into clumps. This reaction is used in the diagnosis of typhoid fever. The serum obtained from the suspected case is tested in various dilutions—50 per cent., 5 per cent., and 0·5 per cent.—and the effect noted in half an hour. The dilutions are made by

diluting the serum with sterile broth by means of a capillary pipette, and then mixing equal loopfuls of typhoid culture and serum.

Two pipettes are required: a 90 cmm. and a 10 cmm. The dilutions are made as follows :—

(1) 50 per cent.: one loopful of the broth culture is mixed with one loopful of the serum to be tested on the coverslip.

(2) 5 per cent.: 90 cmm. of sterile broth are mixed with 10 cmm. of the serum, and a loopful of the mixture of serum and broth added to a loopful of the broth culture on the coverslip.

(3) 0·5 per cent.: 90 cmm. of broth are added to 10 cmm. of the 10 per cent. solution; and equal loopfuls of this and the broth culture mixed on the coverslip.

By the above method the manipulation of the cultivation is limited to the platinum loop.

The culture used should be a twenty-four-hours-old broth cultivation, and a time limit of half an hour observed in estimating the reaction.

(2) *Lysogenic Action. Pfeiffer's Reaction.—Bacteriolysis.*

The culture of the organism to be tested is mixed with the immune serum, and the mixture injected into the peritoneal cavity of a healthy animal. Small quantities of fluid are removed from time to time for observation; the organisms are observed to become swollen, contorted and finally dissolved.

If an animal A be immunized to another's (B) red blood corpuscles by the injection of increasing doses of washed red discs, the serum of the injected animal (A) will eventually be found to cause laking (hæmolysis) of the red corpuscles of B when serum A is added to the blood of B in a test tube at body temperature.

(3) *Inhibition of Growth. Wright's Method.*—The cultivation of the organism to be tested is mixed with various dilutions of the serum and melted gelatin. The mixture is introduced into capillary tubes and the presence or absence of colonies noted after incubation. The immune serum prevents the development of the corresponding organism. Control experiments without serum addition should always be made.

We are now in a position to review the various theories of immunity that have been advanced from time to time to explain the foregoing phenomena.

Pasteur suggested the theory of exhaustion, by which he endeavoured to explain the production of immunity as due to the

using up by the infecting organisms of some portion of the tissues especially adapted to their growth; and that when this hypothetical proteid fragment was exhausted the organisms could no longer live or find substances fitted for their activity, and therefore gradually perished.

This supposition is entirely negatived by the production of passive immunity by the injection of antitoxic sera, which can hardly exhaust the tissues of the injected animal.

The Theory of Retention supposes that products inimical to the bacteria present are retained in the animal body, and that due to their presence the organisms die out, just as they do in an old cultivation in a test tube. Such a suggestion is, however, at variance with the main facts given above, and is also opposed to the fact that death of an animal can take place by an overdose of toxine, although its serum is antitoxic for other animals.

Phagocytosis. — Metchnikoff, who first advanced this theory, which is particularly related to the question of natural immunity, supposes that certain cells of the blood, termed phagocytes, especially function as destroyers of bacteria. Of these cells two main varieties are present in the human subject : (*a*) polymorphonuclear leucocytes or microphages, and (*b*) the larger varieties of connective tissue cells endowed with amœboid movement (macrophages). These cells are endowed with the power of ingesting foreign bodies, retaining them in their protoplasm until they are either wholly or partially digested, when if the phagocyte retains its vitality the remains are extruded. Such a phenomenon can be directly observed by keeping amœboid cells of this class upon a warm slide in the presence of bacteria. The bacteria may be watched during the whole process of engulfing, &c., and are to be seen within the plasm of the cells. The phagocytes will be seen to move in the direction of the organisms, impelled probably by the secretions of the bacteria; at other times the cells recede as if to withdraw beyond the influence of a too powerful poison; these movements are severally known as *positive* and *negative chemiotaxis*.

Metchnikoff observed that in a susceptible animal these phagocytic movements were slower, more ill-defined, or were absent, while in an animal possessed of a high degree of immunity the cells were relatively more active and ingested the bacteria with apparent ease and avidity; moreover, he also observed that the cells of a susceptible animal acquired greater power of dealing with

bacteria by this process of phagocytosis in proportion to the degree of immunity conferred by artificial means, in fact as immunity increased so did the phagocytic power of the cells. Natural immunity, therefore, would be due under this conception to a relatively robust and active state of the phagocytes, while susceptibility would result from a slow and indifferent phagocytic power. The phagocytes have, as it were, become more and more educated in the hunting of bacteria.

It is, however, difficult to entirely reconcile the facts given above with the production of immunity by the injection of toxines without the bacterial bodies themselves. That a large number of bacteria are destroyed by the phagocytes is undoubted, and, moreover, in certain diseases in which bacteria are present in the blood a marked phagocytosis is developed, but the presence of such an extra development of white blood cells appears to be more in the nature of a concomitant phenomenon than the means by which immunity is effected.

Natural Immunity.—We have so far considered the question of immunity from the standpoint of the pathological phenomena concerned in the resistance and susceptibility to various disease-producing bacteria. The number of pathogenic bacteria is, however, small when compared with the numerous species which exist as saprophytes, and which do not, even when introduced into animals, produce symptoms of poisoning.

But animals are not always susceptible to even pathogenic organisms, for instance, the common fowl is highly resistant to tetanus, the common mouse is immune to tubercle, while the field mouse is susceptible, the guinea-pig is resistant to the pneumococcus, while the rabbit is peculiarly susceptible, and so on.

In some cases it has been shown that the animal naturally immune has some antitoxic power in its serum, and that the blood is also antibacterial, but this is only in a few cases and is not sufficiently generalised to account for the immunity possessed by many animals.

Animals, therefore, possess a natural immunity to certain diseases which may be due (*a*) to the power of animal tissues generally to destroy bacteria (*b*) to the ease with which the toxines of the infecting organisms are neutralised in the body. Both of these processes may be in operation at the same time and by appropriate means one or other may be so lowered by artificial

means that the animal easily develops the disease. Thus Pasteur found that the fowl, normally immune to anthrax, became susceptible when its body temperature was reduced by cold water. Guinea-pigs and rats kept in an atmosphere of sewer air showed lowered resistance towards diphtheria bacilli. Animals, such as rats, &c., which have been fatigued by exercise in revolving cages, are found to be much more susceptible to staphylococcal injections than control animals.

Immunity is therefore in some way concerned with the normal functions of the body, but at present very little is known of the subject. It is probable that fresh light will be thrown on the subject by the large amount of enquiry that is at present going forward relative to the anti-bodies produced during artificial immunization, and it is also possible that the property of natural immunity to many diseases is the expression of a gradually developed tolerance to the attacks of micro-organisms evolved over long periods of time, and produced in a manner analogous to artificial immunization. Immunity is, moreover, transmitted from mother to offspring.

CHAPTER VII.

Pathogenic Bacteria of the Mouth.

From time to time various pathogenic bacteria are to be found inhabiting the mouth, and may be obtained from the fluids of that cavity, sometimes by means of cultures alone, but at any rate with certain species, best by the inoculation of animals with saliva.

Most of the bacteria thus obtained prove to be members of well known species which are generally associated with disease and pathological conditions in other regions of the body, a few are members of species as yet little studied, whilst a good many described by various authors have occurred in isolated cases only. It is impossible, in many cases, to find an adequate description of many of this latter series, and the task of connoting all the evidence is particularly difficult; some of the organisms may well belong to known species, modified perhaps by their residence in the mouth in such a manner as to render them difficult to identify in the first cultures obtained.

In noting some of these bacteria in the present chapter, the description given by the observers who originally described them have been rigidly adhered to, and the *whole* of the data obtainable given. This has been done with two reasons: firstly, to make the chapter as complete as possible; and secondly, by collecting the various scattered researches to enable other workers to confirm or disprove the various statements made. Special note is made of any of these organisms I have met with myself during a somewhat extended series of researches in the last seven years. Many of the pathogenic bacteria which occur in the mouth are apparently living in a state of œco-parasitism, ready under favourable circumstances and environment to act as the liberating impulses of disease.

Amongst the most commonly present the pneumococcus is perhaps the most frequent; its pathogenicity varies within wide limits. It is somewhat interesting to note that this organism, discovered independently by Fränkel,[1] Pasteur[2] and Sternberg,[3] was first

[1] *Zeit. für Klin. Med.*, 1885.

[2] *Compend. rend Acad. des Soc.*, *Paris*, xcii., p. 159-165.

[3] National Board of Health Bull., vol. ii., 1882.

isolated and described by inoculating rabbits with saliva. Pasteur was searching for the organism associated with rabies, Sternberg for the cause of yellow fever.

Another pathogenic organism whose presence in the mouth is more general than is often appreciated by dental surgeons, and which appears in about 33 per cent. of all persons exposed to infection, is the Klebs-Lœffler or diphtheria bacillus, and moreover the spread of this disease is largely due to the transference of the organism from one individual to another, more particularly children. So much is this the case that notwithstanding the increase of sanitary knowledge and the application of general principles of hygiene to everyday life, the disease, once more common in rural than urban districts, has now become a disease more associated with town than country areas, and shows a most striking relationship to the progressive aggregation of children for educational purposes. It is therefore a disease which should be borne in mind by all dental surgeons. In all mouths, and with no exception as far as I can ascertain, a streptococcus is a normal inhabitant, but apparently exists as a distinct species of a non-virulent type, although at times true pathogenic streptococci are to be met with. B. coli communis is also to be found rarely, while the tubercle bacillus may be found in the subjects of tuberculosis of respiratory tract. The bacillus of blue pus, B. pyocyaneus, is found in a limited number of cases, as is the Micrococcus tetragenous and rarely the Streptothrix actinomyces. These more important pathogenic organisms will be described first and following them a second group, comprising pathogenic organisms observed and described by various authors as peculiar to the mouth.

(1) STREPTOCOCCUS PYOGENES.

This streptococcus is the organism associated with erysipelas and general pyæmia. It has been found also in a large number of other pathological conditions, such, for instance, as infective endocarditis, puerperal septicæmia, acute infective periostitis, &c. Associated with other pathogenic organisms it is sometimes found in diphtheria, bronchitis, pneumonia (especially the type known as "septic pneumonia"), as a secondary infection in phthisis, &c. In many cases of obscure febrile type the organisms are to be obtained from the circulating blood.

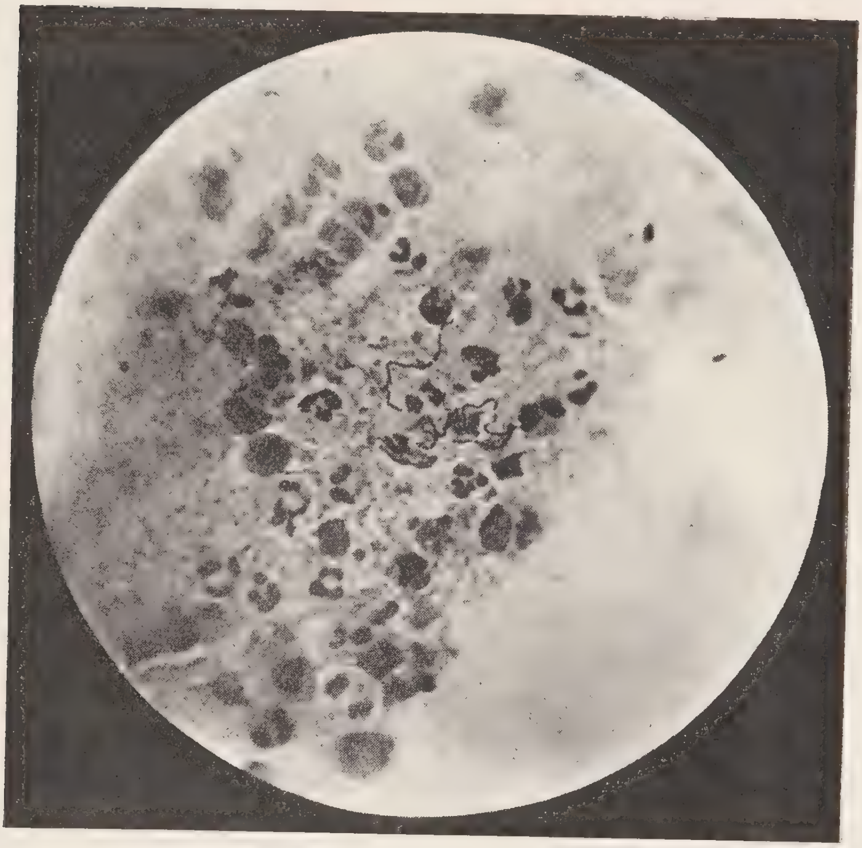

FIG. 32.—STREPTOCOCCUS PYOGENES IN BLOOD. × 1,000.

FIG. 33.—STREPTOCOCCUS PYOGENES.

Twenty-four hours' agar cultivation. Stained Gram. × 1,000. (From Washbourn and Goodall's "Infectious Diseases.")

The streptococci are among the most widely distributed of the pathogenic cocci, and are as a general rule present in all the more acute suppurative conditions found in man.

Varieties.—Formerly the streptococcus of phlegmonous erysipelas, and the S. pyogenes were considered different species, but from a series of extended researches it is now generally admitted that the two species are identical.

FIG. 34.—STREPTOCOCCUS PYOGENES.
Twenty-four hours' agar cultivation. $\frac{2}{3}$.

Petruschky placed this beyond doubt, by obtaining a virulent streptococcus from a purulent peritonitis in a patient never having suffered from erysipelas. He then inoculated the cultures into two individuals suffering from cancer, who had never had erysipelas, and produced a definite attack of erysipelas.

Linglesheim,[1] by a long series of carefully-conducted researches,

[1] *Zeitsch. für Hygiene*, Bd. x., p. 331.

came to the conclusion that the streptococcus normally resident in the mouth was of a distinct species to the organism causing disease.

Other observers, however, have held that all streptococci, wherever obtained, are simply different races of the pathogenic streptococcus, and may be raised to the same standard of virulence by appropriate means. This question is considered more at length below, and I will only remark in passing that it cannot be definitely stated that all the streptococci found occurring in disease are of the same species.

Morphology.—Cocci, 0·4—1 μ in diameter, generally united in chains of four to twenty or more individuals, the longer chains are generally formed in liquid media. When rapid division is taking place the appearance of a chain of diplococci is produced. According to Sternberg, the cocci may occasionally occur as diplococci in culture media. Sometimes the cocci become so much elongated that the appearance of bacilli is produced.

In old cultures particularly, some of the elements of the chains appear enlarged and swollen and greatly exceed the size of the rest of the cocci in the chain; the bodies have been termed arthrospores by Hueppe and Du Bary, and are thought to be more resistant than the purely vegetative forms. Other bacteriologists consider them to be involution forms. No true endospores are formed, the cocci are not motile and do not possess flagella.

Staining Reactions.—Stains well with the ordinary aniline dyes and by Gram's method. Old cultures, and especially cultures on potato, show irregular staining.

Biological Characters.—An äerobic, facultative anäerobic, non-motile pathogenic streptococcus. Spore formation not known, not motile, no flagella present, does not liquefy gelatin. Optimum temperature 37·5° C., but will grow at 16° to 18° C. Thermal death point 54° C. for ten minutes.

Gelatin Stab, 22° C.—Growth slow; in three days a line of small spherical, translucent, whitish colonies, giving a beaded appearance. Surface growth weak, flat, and edge entire. No liquefaction occurs.

Gelatin Streak, 22° C.—A series of small, flat, whitish colonies, rarely forming a continuous streak.

Gelatin Shake, 22° C.—A cloud of minute spherical colonies are seen distributed throughout the medium in three to four days. No gas is formed.

Agar, 37·5° C.—A series of grey translucent flat colonies develop along the streak, in places becoming confluent. The edges of the colonies when observed with a lens are seen to be composed of loops of chains of cocci.

Blood Serum, 37·5° C.—Minute grey-white colonies similar to agar.

Potato.—Little growth occurs, and in forty-eight hours at 37·5° most of the cocci have become swollen and involuted.

Broth.—Development somewhat slow at 37·5° C. The fluid remains clear, and numerous thin flat flocculi form, which tend to settle to the bottom or to the inclined side of the tube; when blood serum or ascitic fluid is added to the broth, development is more rapid. A faint acid reaction does not prevent the growth, although the organisms grow best in an alkaline or neutral medium. No indol formed.

Glucose Broth.—Acid, no gas.

Litmus Milk, 37·5° C.—Well marked acid reaction in two days, later the milk may be coagulated, but this is by no means constant.

The vitality of the cultures is not great on liquid media. They are best kept on solid media, and withstand drying for some time.

Pathogenesis.—The streptococcus varies considerably in the amount of pathogenic power possessed by various races, and is not, as generally obtained, very virulent for animals. Mice and rabbits, if inoculated with virulent culture, die in twenty-four hours to six days, of general septicæmia, the organisms are present in large numbers in the heart blood and in the various organs. If the culture be less pathogenic, disseminated or metastatic abscesses are found distributed about the various organs.

Marmorek[1] has shown that the initial virulence possessed by streptococci obtained from various pathological conditions arising in the human subject, may be raised to a great degree of virulence by the rapid passage through rabbits, one animal being inoculated with the blood of another just dead of streptococcal infection. The cultures were made upon a special medium, consisting of three parts of human blood serum to one of broth.

Bulloch[2] also produced enormous exaltation of virulence by passage through rabbits. The culture on ascitic fluid-broth, which originally had a M.L.D.[1] of 0·1 ccm., was increased in virulence to

---

[1] *Ann. de l'Inst. Pasteur*, t. ix., No. 7, 593.

[2] *Lancet*, April 11, 1896.

such an extent that 0·000001 ccm. was the M.L.D. Many other workers have confirmed these results. Widal and Besançon found that a streptococcus which originally possessed no virulence became pathogenic when inoculated with Bacillus coli communis, and that subsequent cultures could be raised in virulence by Marmorek's methods. These observers also found that the streptococci obtained from the mouth of a small-pox patient were non-virulent, whereas those present in the blood exhibited a considerable degree of pathogenic power.

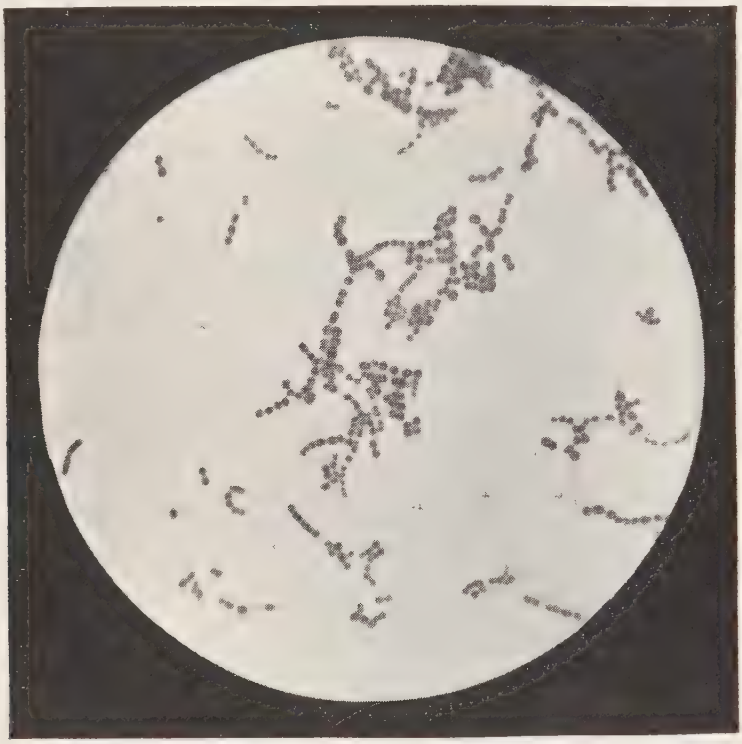

FIG. 35.—STREPTOCOCCUS PYOGENES.
From twenty-four hours' broth cultivation. Stained Gram. × 1000.

(2) STREPTOCOCCUS OF THE MOUTH.
(*Streptococcus brevis.*)

Any one who has made cultivations from the mouth cannot but have been struck with the frequency of streptococci in the cultures.

In all mouths, healthy or unhealthy, clean or dirty, I have never failed to obtain the streptococcus. Not only is it present in the mouth proper, but it exists in the antrum of Highmore, in the Eustachian tube, nose, and middle ear. In these situations it occurs typically as diplococci massed around the dead squamous

[1] Minimal lethal dose.

FIG. 36.—STREPTOCOCCUS BREVIS OF MOUTH, MASSED ROUND EPITHELIAL CELL, SHOWING DIPLOCOCCAL FORM.
Stained Gram. × 1,000. (Washbourn and Goadby, *Trans. Odont. Soc.*, 1896.)

FIG. 37.—STREPTOCOCCUS BREVIS.
Agar cultivation at twenty-four hours. Stained Gram. × 1,000

epithelial cells (fig. 36). It is by no means confined to the human mouth, and I have observed it in the mouths of monkeys, dogs, rabbits, and guinea-pigs.

The diplococci form of this streptococcus can be easily seen in almost any coverslip preparation made from the mouth direct. A cover glass specimen is made by smearing saliva, scraped from the buccal sulcus with a platinum loop, on to the cover glass. The film is allowed to dry and then stained with carbolic-methylene blue or other stain. The diplococci will readily be recognised massed around and adhering to the squamous epithelial cells, some of the diplococci having the appearance of short bacilli owing to the elongated and somewhat pear-shaped form of the cocci. The question of the identity of these diplococci with the streptococcus may be proved in the following way: clean coverslips are smeared with melted agar to which a little saliva containing some epithelial cells has been added; when dry, the small coverslip-plate is cemented to a hanging drop slide (a slide with a glass ring cemented to it, see fig. 13) and fixed in place with a little canada balsam. The preparation is then placed on the stage of the microscope and an epithelial cell sought for with the diplococci attached. The microscope with the preparation *in situ* is then placed in the incubator at 37·5° C. for twenty-four hours. At the end of this time the preparation is examined, when the diplococci will be found to have developed into colonies which surround the cell and in which the streptococcal chains may be easily seen. With a little care one colony can be selected and marked, and cultures on broth and agar made from it as well as coverslip preparations. The culture tubes will show a good growth of streptococci.

This method of obtaining a pure culture of the mouth streptococcus is somewhat tedious, and the method adopted by Dr. Washbourn and myself is much less difficult. Broth cultures are made by adding a loopful of saliva to a tube of nutrient broth; the tube is then incubated for twenty-four hours, at the end of which time the tube will contain an impure culture of streptococci and other organisms. An agar tube is now inoculated from the broth tube and the agar tube incubated at 37° C. In eighteen to twenty-four hours the sloped surface of the agar tube will be found to be covered with a number of small grey-white colonies, which, transferred to another tube, will give a pure culture of the mouth streptococcus. A little care must be exercised in picking out the colonies,

as other organisms are often also present, but there is generally no difficulty in obtaining a pure culture. The explanation of the ease with which a culture can be obtained is that the mouth streptococcus grows with great rapidity in almost all media, to the exclusion of other organisms.

The streptococcus obtained from various mouths often differ slightly in their cultural characters, but in their general behaviour they conform to the type of von Lingelsheim's[1] Streptococcus brevis, as noted by Dr. Washbourn and myself.[2] Since that paper was published I have obtained the streptococci from 150 consecutive mouths examined; all the cultures obtained conformed to the general characters given below. All of them produced an acid reaction when grown in carbohydrate media (broth to which 2 per cent. of lactose, maltose, glucose, dextrin, cane sugar, or starch had been added). The starch and cane sugar media require the longest to develop the acid reaction, whilst in the other solutions the reaction is often strongly acid in six hours.

The streptococci also clot milk into a solid mass in forty-eight hours.

In the paper quoted above it was noted in connection with the mouth streptococcus that among other characters the few inoculation experiments performed confirmed von Lingelsheim's view that the mouth streptococcus differed from the Streptococcus longus in not being pathogenic for guinea-pigs, rabbits and mice. Other observers are inclined to the view that the streptoccocus of the normal mouth is the ordinary pathogenic streptococcus which occurs in pyæmia and phlegmonous erysipelas. Pathogenic streptococci undoubtedly do occasionally occur in the normal mouth, as various observers have shown—a fact that considerably complicates the problem. The pneumococcus occurs in normal human saliva in a distinctly pathogenic condition, so much so that a rabbit, an animal particularly susceptible to the pneumococcus, often dies subsequent to an inoculation with saliva, the pneumococcus being found in the blood after death. One of the races of pneumococci obtained from the saliva by Washbourn[3] and Eyre were, however, apparently living in a saprophytic condition, and

---

[1] *Zeitschrift für Hygiene*, Bd. x., p. 331.
[2] *Trans. Odont. Soc.*, June, 1896.
[3] *Brit. Med. Jour.*, Nov. 4, 1899.

their virulence was of low value until the organisms had been pssaed through the bodies of many animals ; even then the pathogenicity of the species soon ran down when grown upon artificial media, whilst other races of pneumococci obtained from the rusty sputum of pneumonia retained their virulence for a considerable time. Virulent diphtheria bacilli may be present in normal mouths as well as other pathogenic organisms, and we may certainly also conclude that the streptococci of a pathogenic nature met with in the mouth from time to time are stray individuals of another species accidentally present, and not the common mouth inhabitant. This question of the identity of two presumably different organisms is a much wider question than the particular case of the mouth streptococcus ; thus, for instance, B. coli communis and B. typhi abdominalis, B. diphtheriæ and the B. of Hoffmann, B. subtilis and B. anthracis, to mention only a few examples, are each related to the other in their cultural peculiarities, method of staining, &c., and somewhat minute differences are relied upon to differentiate the organism from its simulator. It is of course possible that the streptococcus of the mouth is a degenerate non-pathogenic and saprophytic variety of the Streptococcus longus, and that under some favourable conditions it may invade the tissues, as in severe scarlatinal angina, and produce serious results. On the other hand the mouth streptococcus may be a different species, having certain characters in common with the Streptococcus longus it is true, but differing from it in others, among which is its virulence. Lingelsheim[1] was the first to point out that the streptococcus obtained from the normal mouth differed from the Streptococcus longus. Thus it was not pathogenic for rabbits or mice, it rendered broth uniformly turbid, and the chains on this medium were shorter than those of the Streptococcus longus and it caused a slight liquefaction of gelatin, and Lingelsheim therefore considered it a distinct species and named it the Streptococcus brevis, from the short chains formed on broth cultures. Marmorek,[2] in opposition to this, looks upon all streptococci as simple varieties of the same species, which can all be raised to a uniform type by appropriate means, although various strains of streptococci obtained from different pathological conditions of the human subject behave differently when injected into animals.

---

[1] *Loc. cit.* [2] *Loc. cit.*

In the paper already quoted[1] the following conclusions are given :—"The streptococcus occurring in the normal mouth agrees with the S. brevis of Lingelsheim, and can be distinguished from the streptococcus of disease by its biological and morphological characters. It must be looked upon as a distinct species for the present, although ultimately this view may prove to be incorrect, for it is possible that further researches may enable us to convert the Streptococcus brevis into the Streptococcus longus. This, however, has not hitherto been accomplished. We think that the discrepancies of different observers who have investigated the question are partly due to the fact that the pathogenic Streptococcus longus is sometimes accidentally present and has been mistaken for the normal streptococcus of the mouth."

Subsequent research tends to confirm these conclusions, and for the present the S. brevis of the mouth is to be regarded as a distinct species and as the most constant of all mouth organisms. In perfectly clean and healthy mouths it is often the only organism met with.

Various other pathogenic organisms have been stated to be present in the normal mouth. Biondi[2] particularly gives a list of five organisms of this class, which require notice. Of the five organisms in Biondi's list two were only met with once, in each case by inoculating an animal with saliva, the organism being found in the resulting abscess. These two organisms then (**Coccus salivarius septicus** and **Staphylococcus salivarius pyogenes**) can hardly be called true mouth bacteria.

The third on Biondi's list (**Streptococcus septo-pyæmicus**) is said to be indistinguishable from the streptococcus of pyæmia and erysipelas in its cultural peculiarities and its pathogenic action on animals, and there seems no reason to doubt that this streptococcus was the S. pyogenes which we have seen is at times present in the mouth.

The fourth organism described by Biondi is the **Micrococcus tetragenous**, whilst **B. salivarius septicus** has since been shown to be the same as the Diplococcus pneumoniæ.

---

[1] *Loc. cit.*

[2] *Zeitschrift fur Hygiene*, Bd. ii., 1887, p. 194.

(3) STAPHYLOCOCCUS AUREUS.

The Staphylococcus aureus, or golden coccus, first carefully described by Rosenbach in 1884, is commonly present in suppuration, abscesses (acute), boils, carbuncles, osteomyelitis, ostitis, &c., and occasionally in puerperal fever, infective endocarditis and pyæmia. In cultures from septic throats it is often present and is commonly found as a contamination in cultivations made from the throats of persons ill with diphtheria. Outside the body it may be found in air, soil, or water, but most frequently in dust, especially the dust of hospital wards.

The staphylococcus may occur in the eyes, ears, nose, mouth, and especially under the finger nails, whilst they are said to occur occasionally in the fæces of children.

I have several times obtained the staphylococcus aureus in pure cultivation from acute abscesses involving the roots of "dead teeth," but it is by no means always present, for in forty cases of oral suppuration around teeth they were only present three times, twice in pure culture. On two occasions I have found a pure culture of staphylococcus aureus in antral suppuration, but even in this region it is far from common.

In neglected and dirty mouths these organisms are occasionally to be found, in healthy mouths they are not often found.

The majority of observers agree that the S. aureus is by no means always present in the mouth, thus: Netter[1] only found S. pyogenes aureus in seven out of 127 individuals examined; Vignal[2] and Miller[3] only found the pyogenic cocci occasionally; Black,[4] on the other hand found S. aureus in seven out of 10 cases examined. My own researches tend to confirm those of Netter and Miller, the S. aureus occurring in about 10 per cent. of all cases examined. On looking through the notes of the examination of 1,000 mouths, I find staphylococcus albus occurs eighty times, about 10 per cent. In these cases there was no special search made for the organisms, but the yellow colonies were observed in the ordinary process of cultivation, isolated and grown on the various test media.

A fact that may to some extent explain the rare occurrence of the staphylococcus aureus in the mouth is the bactericidal power of

---

[1] *Revue d'Hygiene*, 1889, No. 6.

[2] *Arch. de Physiol. normal et path.*, 1886, No. 8.

[3] "Micro-organisms of the Mouth," p. 265.

[4] *Trans. Ill. St. Dent. Soc.*, 1886.

the saliva experimentally proved by Sanarelli. Sanarelli[1] filtered saliva through a Pasteur-Chamberland filter and tested the filtrate in the following way :—

A small quantity of a pure cultivation of staphylococcus aureus, just as much as could be taken up on the point of a platinum needle, was added to 10 cubic centimetres of saliva sterilised by filtration. After mixing, plate cultivations were made, and the number of organisms present determined. Plate cultures were then made at intervals, and by this means it was found that the number of colonies developing gradually diminished, and in thirty-six hours no staphylococci were present.

If, however, a whole loopful of the cultivation was added, the plate cultivations still showed a diminution in the number of organisms present during the first two days, but after this the number again increased, until the colonies were uncountable.

It follows, therefore, that the saliva possesses a certain bactericidal power which is able to deal with small quantities, or isolated organisms, but that this power is quantitative, and that it is insufficient to deal with a large number. In this respect the action is similar to that of blood serum and other body fluids.

It is interesting to note that pneumococci were apparently not affected by the action of the saliva, and grew from the first.

Morphology.—Round, spherical or oval cocci, 0·6—0·9 μ diameter, occurring in clusters or singly and in chains, showing a slight flattening at the point of mutual apposition. Not possessed of flagella. According to Du Bary and Hueppe arthrospores are formed ; these consist of large swollen elements 1·0 to 1·5 μ wide, which stain deeply, other observers are inclined to regard such forms as degenerate cocci, and they are known also as "involution forms." No endogenous spore formation is known to occur. Capsule not stainable.

Staining Reactions.—Retains the stain of Gram's method, and stains easily with the ordinary basic aniline dyes, carbol methylene blue, gentian violet, &c.

Biological Characters.—Non-motile, äerobic, facultative anäerobic, chromogenic coccus, with well marked fermentative and pathogenic powers.

Gelatin, 22° C.—Plates in forty-eight hours white punctate colonies, which rapidly liquefy the medium, microscopically granular, dark brown, entire.

---

[1] *Centr. für Bakteriol.*, Bd. x., 1891, p. 817.

Gelatin Stab, 22° C.—Growth to bottom of stab with rapidly following liquefaction to depth of puncture, cone-shaped liquefaction, with thick golden-yellow precipitate and thick flocculi in fluid.

Gelatin Streak, 22° C.—Well marked groove of liquefaction in three days with flocculent yellow precipitate.

Agar Streak, 37·5° C.—Moist irregularly crenate, raised, yellow. Pigment formed at 37·5° C., but best at 22° C.

Agar Stab, 37·5° C.—Growth to bottom of puncture.

Potato.—Yellow, moist and well marked development at 37·5° C. and 22° C., most pigment at 22° C.

Blood Serum (*coagulated*), 37·5° C.—Similar to agar, but colonies may be smaller, no liquefaction occurs.

Broth, 37·5° C.—General turbidity in twenty-four hours at 37·5° C., a heavy yellow-brown precipitate gradually settles, often in flakes.

Litmus Milk, 37·5° C.—Acid reaction in twenty-four hours, coagulation of casein in two to four days with subsequent digestion of coagulum.

Peptone Water, 37·5° C.—Similar to broth. All cultures give off a sour acid smell. Optimum temperature, 37·5° C.

Carbohydrate Media—Well marked acid reaction. The liquefying enzyme may be separated from the cultures by precipitating with alcohol, and will produce liquefaction independently of the organisms. The thermal death point of this organism is between 56° and 58° C. for ten minutes, when in the moist condition; when dry, however, a temperature of 90° to 100° C. acting for the same time is required.

The resisting power to antiseptics has been tested by a large number of observers: Gärtner, Berhing, Tarnier, and Vignal, all agree that the organism is destroyed by exposure to 1:1,000 mercuric chloride for one to three minutes. Abbot,[1] however, has shown that in the same culture all the cocci are not of the same resistance, and that occasionally there may be forms that will resist an exposure of ten, twenty, or even thirty minutes, although the majority are destroyed by the same solution in two minutes.

Chromogenesis variable, some cultures giving a marked colour, others only a faint yellow. After some time at 37·5° C. the colour tends to become a dirty buff.

---

[1] *Bull. Johns Hopkins Hosp.*, vol. ii., 1891.

A variety of S. pyogenes aureus which does not liquefy gelatin is said to exist, the colour being darker and less brilliant.

Pathogenesis.—Well marked virulence in some specimens, but variable and often lost in saprophytic condition.

Garré caused a well marked carbuncle on his own arm by applying a virulent culture of S. pyogenes aureus to the uninjured skin.

Bumm produced abscesses by inoculating the cocci suspended in normal saline under his own skin.

Becker and others found that by injecting the staphylococcus into the circulation of animals after fracturing or crushing a bone, osteomyelitis resulted.

Ribbert and Rosenbach[1] produced ulcerative endocarditis by injecting the cocci into the circulation of animals whose cardiac valves were injured by passing a sound down the carotid artery; many other observers have confirmed these experiments.

The injection of small quantities of staphylococcus aureus under the skin of animals may often produce no effect; fairly large quantities, however, generally produce a marked localised abscess which eventually clears up. The injection of small quantities into the blood stream (intravenous injection) generally produces death in rabbits and guinea-pigs, the most characteristic lesions occurring in the cortex of the kidney, where rows of small abscesses, observable by the naked eye, occur. These abscesses may clear up without death of the animal, and Morse[2] has shown that in such cases, besides the plugging of the small vessels with masses of cocci, an interstitial growth of connective tissue has occurred (interstitial nephritis) which may be reproduced by injection of the toxines of the organism. It certainly appears from Morse's paper that some amount of toxine development by the staphylococci occurs in the tissues, leading to development of fibrous tissue.

In septic wounds in man it is often possible to obtain a culture of the staphylococci from the blood, although a general pyæmia rarely results from infection with this organism, but kidney infection and metastatic abscesses of joints may occur. There is generally little difficulty in obtaining cultural evidence of the presence of the staphylococcus aureus in pus or other suspected fluid. A small

---

[1] *Fortsch. der Med.*, 1886, p. 1.

[2] *Journ. Experimental Medicine*, vol. i., 4, p. 618.

amount of the pus should be collected in a sterile pipette (see fig. 38) and a drop or two smeared over the surface of an agar tube; in twenty-four hours at 37° C. well marked development occurs, and the yellow colonies may be easily recognised, isolated and tested on the usual media. Blood is treated in a similar manner, but should be well diluted with sterile broth.

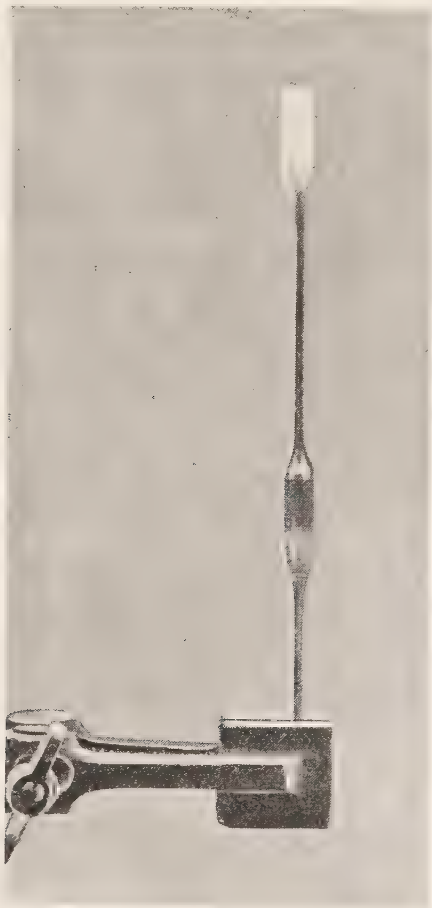

FIG. 38.—PIPETTE FOR COLLECTING PUS.

The top is closed with a cotton wool plug, and the lower end broken at time of use and sealed up in the flame when the tube is filled.

Several other varieties of staphylococci exist, and of these *S. pyogenes albus* is perhaps the most common, and certainly is the most frequently present in the mouth. In the majority of cases of suppuration in and about the mouth this organism may be met with.

In its morphological, biological and general characters the staphylococcus albus closely follows what has been said of the S. aureus with the exception of the production of yellow pigment. Watson Cheyne is inclined to think that this staphylococcus is the most

virulent of the two, but the general opinion is rather of the opposite view.

The S. albus as obtained from the mouth generally exhibit more of the special peculiarities attributed to the S. epidermis albus of Welche in its rate of liquefaction, greater tendency to the diplococcal form, and low pathogenicity.

The *Staphylococcus pyogenes citreus*, another variety of the staphylococcal group, differs from the other two described in the colour of its pigment, which is of a marked lemon-yellow ; it is often present in the mouth, but it is not so virulent as the S. aureus.

Some authors, among them Lehmann and Neumann, are inclined to regard all these pathogenic staphylococci as the same species, and group them accordingly as Micrococcus pyogenes, remarking that the staphylococcal form is not sufficiently constant to form a class term for the species.

(4) DIPLOCOCCUS PNEUMONIÆ (Pneumococcus).

Sternberg in 1880, and Pasteur later in the same year, described a diplococcus of pathogenic power which they had obtained from the saliva of normal individuals.

In the blood of animals injected with saliva a diplococcus was observed, exhibiting amongst other peculiarities a well-defined capsule capable of being stained by appropriate methods. The organisms were extremely difficult to obtain in pure culture ; the use of "blood agar" introduced by Washbourn, however, obviated the difficulty, and the organism may be easily grown on this medium. It is to Fränkel[1] and to Weichselbaum[2] that we are indebted for the discovery of the relationship the organism bears to pneumonia. The pneumococcus or micrococcus pneumoniæ crouposæ, so called from its relation to that special form of pneumonia, is by no means confined to the disease of the lung but has been found in many other situations. As it enters the blood stream and may therefore be carried to any part of the body this is by no means remarkable. The pleural sacs are often directly infected ; a pure culture of the pneumococcus often results

[1] *Zeitsch. für klin. Med.*, Bd. xi., 1886, Heft 5 and 6.

[2] *Wiener med. Jahrbucher*, 1886, p. 483.

from cultivations made from pleuritic effusions. The organism may occur also in the meninges of the brain, setting up pneumococcic meningitis primarily or as a complication of pneumonia; it has been found occasionally in abscesses of the vermiform appendix. The organism has also been observed in suppuration occurring in the subcutaneous tissues, joints, liver, spleen, kidneys, and in otitis media, infective endocarditis, pericarditis, &c.

It occurs with amazing frequency in the saliva of normal individuals. Netter[1] found it present in 15 per cent. of healthy individuals. Wolfe,[2] Claxton,[3] Fränkel,[4] Washbourn and Eyre[5] and many others have also noted its presence in the mouths of perfectly normal individuals. The organism is difficult to cultivate directly from the saliva owing to the presence of other bacteria; the method generally adopted is to inoculate a susceptible animal, such as the rabbit, with saliva. If pneumococci are present the animal dies, and the organisms may be obtained from the heart, blood, and spleen.

The cocci thus obtained differ considerably in their virulence; sometimes the amount of pathogenic power is extremely small, as was the case with the organisms mentioned by Washbourn and Eyre, which required passage through the bodies of fifty-three animals before they attained the same pitch of virulence that another pneumococcus obtained from a case of pneumonia attained after only eight passages. On the other hand, an organism obtained from the mouth is often found to possess a high degree of virulence, and Dr. Eyre informs me that for more than two years fully virulent pneumococci have been present in his own mouth. The organism occurs typically in the rusty or prune-juice sputum of pneumonia and at the edge of the consolidated lung tissue.

Morphology.—Cocci, oval or spherical in shape, generally united in pairs, but also occurring in chains of three or four elements, especially upon agar and liquid media. In the blood of inoculated animals, and in the sputum of acute pneumonia, the cocci are arranged in pairs of oval shape, and surrounded with a gelatinous capsule. The shape of the cocci is frequently lanceolate.

The capsule is said to consist of a substance resembling mucin, and is soluble in water, and does not appear when the organism is grown in artificial media.

[1] *Loc. cit.*
[2] *Weiner med. Blatter*, 1882.
[3] *Med. Times*, Philadelphia, 1882.
[4] *Loc. cit.*
[5] *Loc. cit.*

Staining Reaction.—Stains well by Gram's method and by the ordinary aniline dyes. The capsule may be stained, in fresh specimens from the blood of inoculated animals, or from sputum by means of MacConkey's capsule stain, or by the glacial acetic acid method. Glacial acetic acid is poured on the film prepared in the ordinary way and immediately poured off, and the slip plunged without washing into aniline-gentian-violet.

Biological Characters.—Aerobic facultative anäerobic, non-liquefying, non-motile pathogenic coccus.

Gelatine Plates, 22° C.—Round, grey, 1-2 mm. in four days, deep colonies round, entire, slightly granular microscopically.

Gelatine Stab, 22° C.—Thread-like (filiform), later beaded, no liquefaction, little surface growth.

Agar Plates, 37·5° C.—Macroscopically like gelatin. Microscopically, deep, round, lenticular, greyish-black, coarsely punctate; surface round, entire, translucent, finely punctate.

Blood Agar Streak, 37·5° C.—Greyish, punctate, edge entire or slightly dentate. The colonies generally remain separate.

Blood Serum, 37·5° C.—Slimy, transparent, no liquefaction.

Potato, 37·5° and 22° C.—No growth, except occasionally in old laboratory cultures.

Litmus Milk, 37·5° C.—Sometimes an acid reaction, with or without coagulation, but variable.

Broth, 37·5° C.—Faintly turbid with a slight flocculent precipitate.

Note.—The growth on gelatin plates and stab given above is taken from Lehmann and Neumann's "Bacteriology." On the other hand, Dr. Eyre assures me that only the non-virulent, or slightly pathogenic forms with which he has worked grow at all on gelatin, and even then only upon gelatin streaks, never upon plates or stab cultures. Fully virulent forms do not grow at all upon gelatin at 22° C.

Pathogenesis.—A small quantity of an agar culture inoculated into a mouse, rabbit, or guinea-pig produces death in one or two days. Injection of saliva containing the pneumococci, rusty sputum from lobar pneumonia, or a piece of lung tissue from croupous pneumonia produces the same result. At the autopsy signs of general infection are present. At the site of inoculation there is generally a well-marked fibrinous or gelatinous exudate, often half an inch in thickness. The pleural, pericardial, and peritoneal cavities are generally full of fluid. The spleen, liver, lungs and other organs contain the cocci in large numbers. They are also

present in the heart blood and general circulation—in other words, a general septicæmia occurs. The lungs show no distinct pneumonic changes, although Marli has claimed to produce pneumonia in animals by the injection of pathogenic cultures of the pneumococcus into the trachea.

The cocci exhibit the typical capsulated form when stained from the heart blood of an animal dead of pneumococcic infection.

The amount of immunity produced by infection with the pneumococcus is very brief, and although many observers have attempted the isolation of a definite toxine from the cultures of the organism, no success has so far rewarded their efforts ; it is probable, however, that such a toxine does exist in rather minute quantities.

G. and F. Klemperer showed that the serum of immunised animals protected animals against infection with the pneumococcus.

Washbourn has also prepared an anti-pneumococcic serum which will protect against one hundred times the fatal dose of pneumococci.

The pneumococcus is capable of attaining an enormously high degree of virulence, and in the experiments conducted by Washbourn and Eyre this "standard virulence" was 0·000001 of a loop holding 0·5 milligram of culture, this amount invariably producing death in rabbits, and an anti-pneumococcic serum was obtained of such strength that it protected against 1,000 times this fatal dose. Considerable benefit has resulted from the use of this serum in certain cases of pneumonia. Washbourn and Eyre are continuing their experiments with the pneumococcus in several directions, and have recently published further notes upon the Pathology of Pneumococcic Infection.

(5) MICROCOCCUS TETRAGENOUS.

This coccus was originally observed and studied by Koch and Gaffky, who found it in the lung cavities of tubercular persons.

It is often present in normal saliva, and is not infrequently present in dento-alveolar abscesses. Biondi found it three times in fifty cases examined, Miller also has met with it frequently, as have Park, Vangel and Steinhaus.

Morphology.—Cocci spherical and arranged in groups of four, due to division in two planes at right angles to each other. The individual cocci are about 1 μ in diameter ; the whole group is generally surrounded with a gelatinous capsule. The typical

arrangement and the capsule are only constantly present in the tissues of inoculated animals.

Staining Reactions.—Stains by the ordinary aniline dyes and by Gram's method.

Biological Characters.—Aerobic facultative, anäerobic, non-motile coccus, forming tetrads. Does not form spores; no liquefaction of gelatin occurs.

Gelatin Stab, 22° C.—A well marked convex yellowish viscous mass appears on the surface in two to four days; growth occurs along the needle track as white globular colonies which may become confluent, but generally remain distinct. No liquefaction takes place.

Gelatin Streak, 22° C.—Thick grey-white layer, no liquefaction.

Gelatin Plates, 22° C.—Lenticular, irregular and finely granular and irregular under microscope.

Gelatin Shake, 22° C.—Minute colonies, discrete and globular, lenticular.

Agar Streak, 37·5° C.—Well-marked viscous white layer; colonies may remain distinct.

Blood Serum, 37·5° C.—Similar to agar, no liquefaction.

Potato, 22° C.—Well-marked white layer in forty-eight hours, viscous.

Litmus Milk, 37·5° C.—No coagulation of casein. Slight acid reaction.

Broth, 37·5° C.—Thick stringy viscous deposit and general turbidity.

Pathogenesis.—White mice particularly susceptible, a minute quantity of pure culture causing fatal septicæmia; the organism may be recovered from the heart blood, spleen, liver and other organs. The tetra-cocci are well marked in the tissues. Guinea-pigs are more resistant and generally only develop a local abscess. House mice, field mice and dogs are immune.

The organism probably hastens the tissue necrosis in pulmonary tuberculosis.

(6) BACILLUS DIPHTHERIA.

The Klebs-Lœffler bacillus occurring typically in the membranous exudation of faucial diphtheria may also be found in the anterior part of the buccal cavity, and frequently in individuals who exhibit no clinical or pathological signs of the disease the organisms have

been observed in a fully virulent condition. Thus Aaser[1] found the diphtheria bacillus present in 17 out of 895 soldiers in a cavalry regiment. Park and Beebe[2] found that of 330 persons examined at random, 8 had fully virulent bacilli and 24 characteristic but non-virulent bacilli in their throats. Meade Bolton,[3] among 214 persons more or less exposed to the disease, found virulent bacilli present in 41·5 per cent., and the literature teems with similar cases.

It by no means follows that all the persons in whose throats the diphtheria bacilli are found are suffering at that moment from clinical diphtheria. In a large school of 800 children during an epidemic of sore throat and clinical diphtheria [4] I found 33 per cent. of the whole school had characteristic bacilli present in their throats, while only 14 out of the total number of children examined showed clinical symptoms of the disease. In three of the cases in which no clinical symptoms had at any time manifested themselves, the organisms were in a fully virulent condition, causing the death of injected guinea-pigs in forty-eight hours, with all the characters of infection with the diphtheria bacilli. The importance of those œco-parasites lies in the ease with which they may be transferred from one mouth to another until a susceptible individual becomes the recipient, when grave, often fatal, disease may result; it is, moreover, these "bacillusträgende" persons who may come under the care of the dental surgeon and form an unrecognised centre of infection.

Occurrence.—The Klebs-Lœffler bacilli are found most frequently upon the throats of persons suffering from faucial diphtheria, but are also found occasionally in open wounds, causing wound diphtheria, and upon the conjunctiva.

The bacilli have also been found in milk, which is an excellent medium for their development, several epidemics having been traced to contamination of the milk supply from infected persons.

The organisms rapidly die when introduced into water, and they have never been found in samples of water submitted to examination, nor have they been found in sewage, or in drain and sewer air, or in the emanations of decomposing animal or vegetable matter.

The bacilli will withstand drying for several weeks, and may undoubtedly remain in the dust of rooms in a virulent condition.

---

[1] *Deutsch. Med. Woch.*, 1895, p. 357. [2] *New York Med. Record*, xlvi., 1894.
[3] *Med. and Surg. Reporter*, lxxiv., p. 799.
[4] *Trans. Epidem.*, 1900, p. 99.

They are easily destroyed by the action of germicides, and by a temperature of 58° C. for ten minutes.

When grown in a current of air, Fernbach found that the growth was more luxuriant, but the life cycle shortened. The organism will also grow when air is entirely excluded, and is therefore äerobic and facultative anäerobic. The bacilli may be cúltivated upon the ordinary laboratory media, but are morphologically most typical upon coagulated blood serum, the medium largely used for diagnosis.

Lœffler's blood serum gives even better results; this medium consists of blood serum (liquid) 3 parts, glucose (1 per cent.) broth 1 part.

Coagulation and sterilization are carried out as for ordinary blood serum.

Diagnosis.—In the routine examination of suspected throats for diphtheria bacilli, now largely carried out at the public expense in most British towns, a "sterilized swab" (consisting of a wire, the end of which is wrapped round with cotton wool, and kept till use in a sterilized and cotton wool plugged tube) is introduced into the throat and the surface touched with the sterile wool of the swab. Blood serum tubes are then inoculated, incubated and the culture examined in eighteen to twenty-four hours, when the typical bacilli are sought for. If diphtheria bacilli be present, the colonies, at the end of twenty-four hours will be larger and more defined than those of other bacteria present.

Ohlmacher recommends the examination of the culture in five hours, even though no growth be visible, but a negative result by this means would hardly be of sufficient value to obviate a further examination at twenty-four hours; still a positive result obtained at this time (five hours) is certainly of value. Equally good results may be obtained by examining coverslip preparations of the throat membrane direct and by this method the cultural diagnosis may be forestalled in about 30 per cent. of cases, the stain used being Neisser's two solutions.

Varieties.—Two distinct varieties of the diphtheria bacillus are known, one the short variety, usually considered the least pathogenic, and the "long variety" or most virulent; the ends of the long variety are more frequently swollen and clubbed than the short variety. Both varieties form very short rods upon agar, whilst upon blood serum the "long" variety grows out into rods of 5 to 8 μ in length, or even longer.

FIG. 39.—DIPHTHERIA BACILLUS.

Twenty-four hours' agar cultivation. Stained Gram. × 1000. (From Washbourn and Goodall's "Infectious Diseases.")

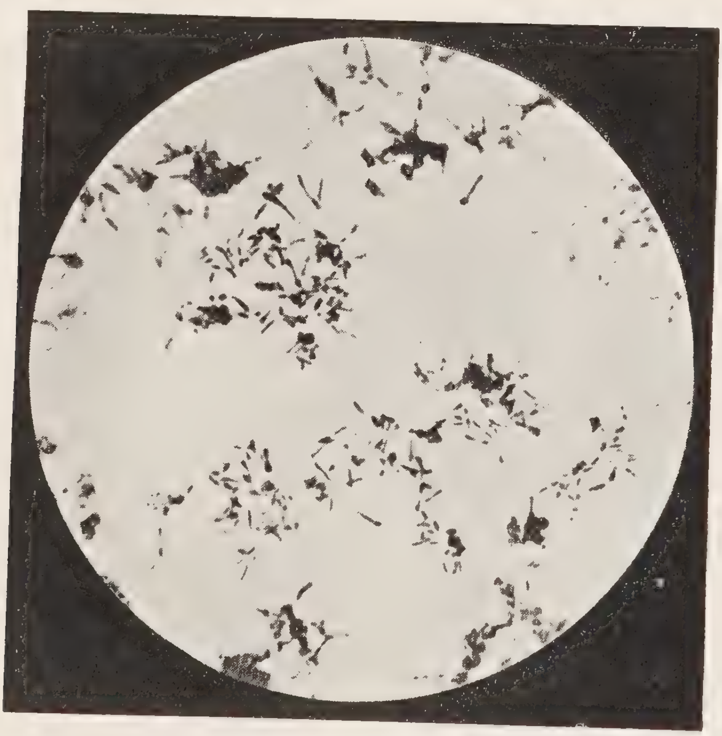

FIG. 40.—BACILLUS DIPHTHERIÆ.

Forty-eight hours' blood serum cultivation. Stained Gram. × 1000.

Morphology.—Straight and slightly curved rods 3 to 4 μ long (twenty-four hours blood serum cultivation), often showing segmentation of the cell plasm by which the bacilli stain irregularly, especially with methylene blue.

By Neisser's method these bands are stained as blue dots, the rest of the organism brown. The bacillus usually lie grouped together with their long axes parallel, constantly the bacilli are of tapering wedge-shaped form, with the bases in apposition.

In older cultivations the ends of the bacilli become swollen and club shaped, forming the characteristic form. Various involution forms occur, the organisms becoming very much swollen, wedge shape, ovoid, &c. The segmentation of the cell plasm is usually well marked. In these older cultures red granules are often to be seen in specimens stained with carbol methylene blue. The organism retains the stain of Gram's method.

Various branched forms have been observed in old cultures as has been also observed with the tubercle bacillus, it is therefore suggested by Hueppe and others that these two organisms are really only a phase in the life cycle of some higher organism allied to the Streptotricheæ, such as S. actinomyces. Chester calls them myco-bacteria.

The diphtheria bacillus is not known to produce spores, although the condensation of the protoplasm and plasmolysis often gives the appearance of sporulation; still the death of the organism at the low temperature of 58° C. precludes the presence of true endogenous spores.

The diphtheria bacillus is not motile and is not known to possess flagella.

Biology.—Growth occurs on the ordinary laboratory media at 37·5° C., and at 22°, the optimum temperature being that of the body. The organism is facultative anäerobic and does not liquefy gelatin, and produces no pigment.

Gelatin Streak, 22° C.—In three days small discrete, raised white colonies, or confluent streak, edge indentate, no liquefaction.

Gelatin Stab, 22° C.—Minute granular, discrete colonies to bottom of stab, no liquefaction.

Gelatin Plates, 22° C.—Minute white points, granular, irregular, under the $\frac{2}{3}''$ granular, irregular and yellowish-brown.

Agar Streak, 37·5° C.—In twenty-four hours does not grow luxuriantly at first, but does so after several transplantations.

Glycerine Agar, 37·5° C.—Delicate moist white to yellowish.

Colonies.—Macroscopical (*a*) *Superficial*, delicate, grey-white, translucent.

(*b*) *Deep*, oval, grey, entire, amorphous.

Microscopical (*a*) Superficial, round, entire, yellowish, translucent.

Blood Serum, 37·5° C.—Opaque white or grey raised colonies, or dull granular moist grey streak.

Potato, 22° C.—Glistening growth on alkaline potatoes which has the same colour as the medium. No growth on acid potato.

Litmus Milk, 37·5° C.—Twenty-four to forty-eight hours acid, no coagulation, later an alkaline reaction appears.

Broth, 27·5° C.—Twenty-four hours granular deposit with fine flocculi, often forming a surface film. Reaction at first acid, later alkaline.

Glucose Broth, 37·5° C.—Acid production. Acid is also formed from glycerine.

Peptone Water, 37·5° C.—Indol produced in seven days. In old cultures some nitrite is also formed, so that a cholera red reaction is given with pure sulphuric acid (nitrate free). A slight amount of sulphuretted hydrogen may be produced.

Pathogenesis. — Inoculation of animals by the subcutaneous method with small quantities of the diphtheria bacilli causes death in from three to six days. Guinea-pigs are the most susceptible, rabbits being considerably more resistant. Subcutaneous inoculation of guinea-pigs with 0·1 to 0·3 cubic centimetres of broth culture results in death. The pathological changes observed at the autopsy are extensive ecchymosis and local œdema at the seat of inoculation, increase of fluid in the various serous cavities, pericardial, pleural and peritoneal; injected, enlarged and hæmorrhagic suprarenal capsules, with occasionally a slight swelling of liver and spleen. There may be a good deal of lymphatic enlargement and congestion, but it is not a constant symptom. Small dotted areas of necrosis and fatty degeneration are often found in the liver, kidney, and heart muscle, more particularly in those cases in which death is long delayed. The most typical lesions are the fibrous-gelatinous exudation at the seat of inoculation from which the diphtheria bacillus can be recovered in pure culture, and the hæmorrhagic suprarenal bodies.

FIG. 41.—BACILLUS DIPHTHERIA.

Blood serum cultivation at thirty-six hours. (From Curtis' "Essentials of Practical Bacteriology.")

The bacillus is not found in the blood or in any of the organs.

Roux and Yersin, who performed a large number of experimental inoculations in demonstrating the undeniable relation of the diphtheria bacillus to the disease of that name, found that rabbits, if inoculated subcutaneously with 2 cc. of virulent broth culture generally died in twenty to twenty-five days. Those animals which remained alive the longest often exhibited paralysis of the hind limbs, and other symptoms recalling the post-diphtherial paralysis of the human subject.

Pigeons generally recovered unless inoculated with 0·5 cc. or more of the broth culture. Rats and mice will withstand large doses and are practically immune.

Toxine formation.—It is clear that a disease such as diphtheria, in which widespread pathological changes are followed by the injection of bacilli, and yet the organisms injected are only to be found subsequently at the site of inoculation, must owe its symptoms to a poison produced by the organisms rather than to the presence of the organisms themselves. That this is the case was first demonstrated by Roux and Yersin, who filtered broth cultivations of the diphtheria bacillus through porous porcelain. The fluid thus obtained is entirely free from bacteria, but contains any soluble poisons produced by the activity of the organism. It was found that the filtered culture, when injected into guinea-pigs and rabbits, produced all those symptoms described as caused by the injection of the bacilli themselves; no bacteria were found, however, at the site of inoculation, although the suprarenal glands showed the typical hæmorrhagic symptoms. The diphtheria bacillus therefore produces a poison or *toxine*.

The formation of the toxine goes on in the broth culture under certain conditions, an alkaline reaction favouring its production, little developing when the reaction is acid. The toxine is destroyed by exposure to the temperature of boiling water, and is much reduced in potency, although not actually destroyed, by a temperature of 58° C. for two hours. It is precipitated from the broth culture by the addition of three or four volumes of absolute alcohol to one of culture, and the white precipitate thrown down is soluble in distilled water. When injected it produces the same symptoms as the injection of broth culture.

Sidney Martin has described a method of obtaining the toxine in quantity, using a solution of alkali albumin to grow the organisms

in, and purifying the alcoholic precipitate by repeated precipitations from water with absolute alcohol. No peptone was added to the solution.

Sidney Martin[1] confirmed the work of Roux and Yersin regarding the enzymic nature of the toxine, and also that of Lœffler respecting the precipitation by absolute alcohol. He separated two chemically different substances from the tissues of persons dead of diphtheria, the one an albumose, the other an organic acid. The intravenous injection of the albumose thus obtained caused paralysis, fatty degeneration, and nerve degeneration (Wallerian) in experimental animals, while the intravenous injection of the organic acid produced similar effects in a lesser degree. Sidney Martin concluded that the diphtheria bacillus produced a toxic enzyme capable of digesting (fermenting) the body proteids and setting free an albumose and an organic acid which caused degeneration of nervous tissue. It is possible that the toxine differs from both these bodies, the precipitated albumoses only carrying down the poison mechanically. Roux and Yersin found that an animal injected with increasing but sub-fatal doses of the diphtheria toxine developed immunity both to large doses of the toxine and to injection with the bacillus itself, and this method is now the one adopted in the production of anti-diphtherial serum. The animal, generally a horse, is injected with increasing doses of diphtheria toxine, the toxine being that produced by a specially virulent organism. When the animal shows no rise of temperature, or other reaction to the injection of large doses of toxine, it is bled from one of the neck veins (external jugular) and the blood received into sterile vessels, allowed to clot, the serum syphoned off and 0·5 per cent. carbolic added, to prevent the growth of moulds.

The serum is then tested as described in the chapter on immunity.

Variations in the Virulence of the Diphtheria Bacillus.—Roux and Yersin found that when the diphtheria bacillus was grown at a high temperature, 39° to 40° C., attenuation of the pathogenic power was brought about; especially was such the case where a current of air was passed over the culture.

Where the virulence had fallen by the above treatment it was found that inoculation of animals with the bacilli and streptococci raised the virulence again.

---

[1] Report L. G. B., 1891-2, p. 170.

They also found that the organisms obtained from the mouth from time to time during convalescence from an attack of diphtheria, underwent a progressive diminution in virulence, and at the same time a change from the longer to the shorter forms occurred.

As a rule the most virulent bacilli are to be found in the most severe cases, but it by no means always follows that the most fatal cases have the most virulent organisms present, the fatality apparently depending considerably upon the resisting power of the individual, as well as the toxicity of the organism. The bacilli may remain present in the mouth for long periods after all clinical signs of the disease disappear and, moreover, frequently retain considerable pathogenic power. Several cases, in which organisms have persisted for upwards of six months, are on record. Those cases in which a nasal discharge persists are generally those in which the bacilli remain the longest.

Pseudo-diphtheria Bacillus (Hoffmann's bacillus).—An organism often found in cases of simple angina, tonsillitis and various membranous varieties of sore throat, much resembles the true diphtheria bacillus in its cultural peculiarities, but differs in the fact that it is not known to be pathogenic. Certain observers have claimed to have produced a virulent form of this bacillus, in other words, to have turned it into the true diphtheria bacillus by passage through the bodies of susceptible animals, but the evidence is by no means conclusive. Curiously enough the Hoffmann bacillus is generally to be found in the latter stages of diphtheria convalescence, but by no means always. Microscopically it is most difficult to distinguish from the short forms of the Klebs-Lœffler bacillus, and for this and other reasons many authorities are of opinion that it is only a non-pathogenic member of the same species, and that the membranous sore throats associated with this organism are in reality a mild non-toxic variety of diphtheria.

The various forms of membranous disease occurring spontaneously in the lower animals are due to other bacteria than the diphtheria bacillus, with the exception of the cat, which has been shown to develop true diphtheria.

There are several other bacteria closely allied to the diphtheria bacillus in their microscopical and other characters, as the xerosis bacillus, and at least three varieties which have been isolated from milk.[1]

---

[1] Eyre, Brit. Med. Congress, 1901.

(7) BACILLUS TUBERCULOSIS.

Tuberculosis is perhaps the most common disease of men and animals, it is chronic in its course in a large number of cases, and the inflammatory reaction by the body cells to the bacterial invasions has many of the characters of a new growth, so much so in fact that tuberculosis, together with syphilis, actinomycosis and glanders, have been classed by Virchow as "Infective Granulomata." The discovery of the tubercle bacillus by Koch in 1882, and fully described, some two years later, in one of the most classical series of researches in the history of pathology, gave an impetus to the study of bacteriology never since lost.

Recently, Koch has stated that the human tubercle bacillus and the organisms affecting cattle are different species and not intercommunicable. The relation of tuberculosis affecting birds to that affecting man has been the subject of considerable research, notably by Nocard, who has come to the conclusion that the two varieties are of the same stock, but the avian bacilli have been modified by their environment.

It would be out of place here to enter into a discussion of all the various lesions of the human subject, or of animals with which the tubercle bacillus is found associated; it is, however, necessary in passing to note the frequency of tubercular invasion of the glands, especially in children, and to point out that the mouth may often act as the portal through which the bacilli enter. It may also happen, in fact so far as one can judge often does happen, that the inflammatory conditions set up in the cervical lymphatic glands by the presence of carious teeth provides a point of lowered resistance in the form of an inflamed gland, and that tubercle bacilli circulating in the blood finding such a spot, settle down and develop, and once established, act as the centre of general infection.

It is also extremely probable that the specific bacilli themselves make their way in from the mouth, along the tracks of engorged and enlarged lymphatic vessels, much in the same way that the cells of an epithelioma so rapidly spread in oral cancer.

The bacilli are not by any means easy to demonstrate in all tubercular lesions, sometimes they are present in large numbers, at other times, as in the discharge from a tubercular sinus, or in the urine of tubercular kidney trouble, they are present only in small numbers and require the most careful search to reveal their presence.

I have not been successful in demonstrating tubercle bacilli in carious dentine.

Morphology.—Bacilli 2·5—3·5 μ in length, 0·3 μ thick. Longer forms, up to 5 μ, are met with. The rods stain irregularly, giving a jointed, or beaded appearance, so much so that the clear interspaces have been described as spores.

At times branched forms are met with, more particularly in the bacilli infecting birds. These branched forms have been described by Hueppe[1] and Fischel as indicating that the tubercle bacillus is really the parasitic form of an organism related to the Streptothrix actinomyces.

The question of spore formation has not been satisfactorily settled, but the spores, if they exist, are much less resistant than the generality of true endospores.

Staining Reactions.—The tubercle bacillus belongs to the series of bacteria known as "acid fast," that is, when stained they resist decolourisation, even with strong acids. The tubercle bacillus has an exceptionally resistant sheath, and therefore does not stain by the ordinary methods. The carbol-fuchsine method of Ziehl-Neelsen, gives the best results, and is employed as follows: The material to be stained, pus, sputum, centrifugalised deposit, &c. is smeared evenly over the coverslip and the film flamed in the ordinary way. The slip is then placed in the stain for five minutes, kept hot over a water bath. After staining the slip is well washed in methylated spirit, until no more colour is washed out, and then rinsed in water. The slip is now immersed for three seconds in 25 per cent. sulphuric acid, rapidly washed in running water, transferred to carbolic-methylene blue for five seconds, washed, dried and mounted. The bacilli are stained red, the tissue and cells blue.

The method is extremely simple, but requires some practice in the decolourising and subsequent double staining.

The bacilli stain by Gram's method, but the plasmolysis of the protoplasm gives the appearance of streptococci.

Biological Characters.—An äerobic, facultative anäerobic, non-motile bacillus, only growing at about 37° C.

Exposure to steam, at 100° C., destroys the organism in two to five minutes (Schill and Kocher). Exposure to a temperature of

[1] *Loc. cit.*, p. 43.

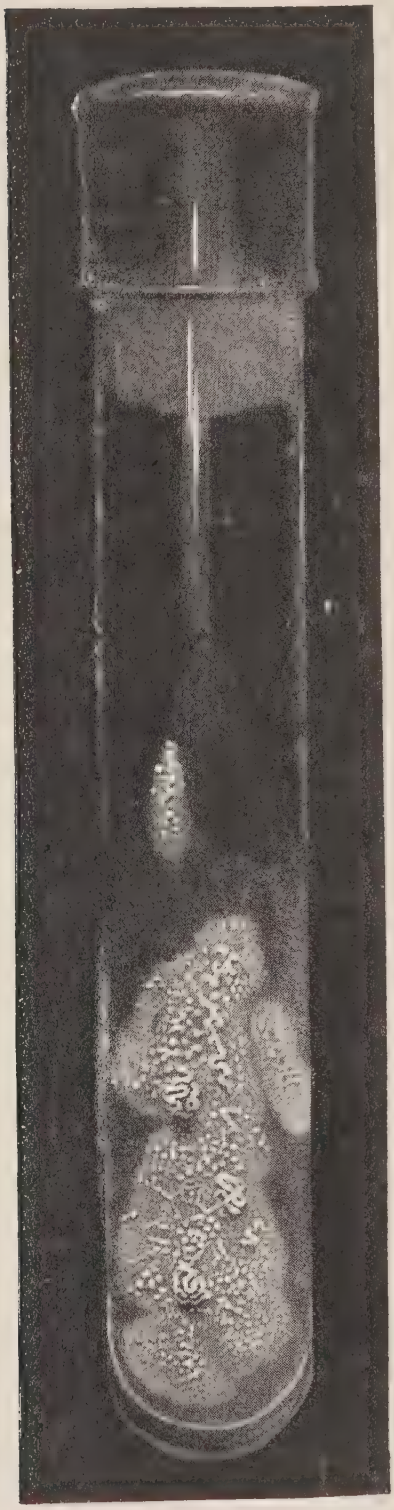

FIG. 42.—BACILLUS TUBERCULOSIS.

Pure cultivation on glycerine agar. Several months old. (From Curtis' "Essentials of Practical Bacteriology.")

60° does not entirely destroy the organisms, whereas exposure to 70° C. kills them at once (Yersin).

Blood Serum.—This medium was the one by which Koch first obtained cultivations. The colonies appear from the tenth to the fourteenth day as minute, irregular, hard, dry points, when obtained direct from tubercular lesions. In subsequent sub-cultures the growth develops more freely and may cover the whole surface, producing a grey, dull, wrinkled, dry layer.

Glycerine Agar, first introduced by Nocard and Roux, is a good medium for the tubercle bacillus. The colonies appear much earlier than serum cultures, but the medium is not a good one for obtaining cultures from tubercular material. The character of the growth is similar to the blood serum.

Glycerine Broth.—Small white masses grow on the surface, and gradually fall to the bottom. If the growth be started upon the surface of the fluid, it gradually covers the entire surface with a wrinkled layer. This method is especially suitable for the production of tuberculin.

Glycerinated Potato.—A wrinkled membrane similar to the agar and blood serum tubes, forms on the surface of the potato; growth will take place as low as 23° C. (Sander).

Pathogenesis.—Subcutaneous injection into animals of pure cultivations, or material containing the bacilli, produces a local swelling in about ten days, and later breaks down, producing a deep cavity with caseous walls. The lymphatics leading from the site of inoculation are enlarged and the lymphatic glands later become caseous. Death may occur in six weeks or be delayed for two or three months. Instead of remaining localised to the lymphatic glands in the immediate neighbourhood of the injection, the bacilli may invade the whole body, setting up a generalised tuberculosis; or deposits may occur in various organs which caseate and break down. The bacilli are to be found in all the lesions if careful search is made. In diagnostic inoculation, if the guinea-pig injected does not die in six weeks, it is killed and a careful bacteriological examination made of the lymphatics draining the inoculated region. Intravenous inoculation produces generalised tuberculosis, intraperitoneal injection tubercular deposits on the peritoneal surface, and in the retroperitoneal and other lymphatic glands. The spleen is commonly affected. Animals may be also infected by causing them to inhale tuberculous dust, and also by feeding with tuberculous material.

Tubercle is common in pigs, cattle, dogs, carnivora, &c. Sheep and goats are practically immune.

Tissue Reaction.—The typical microscopical change produced in invasion by the tubercle bacillus is the *tubercle.* It consists of a central large cell or giant cell, in and around which are to be found the infecting organisms; surrounding this is an area composed of fairly large spindle-shaped cells or epitheloid cells, and outside these again a zone of unnucleated leucocytes.

Action of Dead Tubercle Bacilli.—Prudden[1] and Hodenphyl have found that the injection of sterilised cultures produces tubercles, but that these do not result in a generalised infection, nor do the glands show the presence of the tubercle bacilli. The action is apparently due to the intra-cellular toxines held in the bacterial protoplasm, especially as Stockman has found that animals injected with dead tubercle bacilli give the tuberculin reaction.

Koch's Tuberculin.—Koch found that when a guinea-pig suffering from a tubercular lesion was inoculated in another part of the body with dead cultures of the same bacillus, the caseating gland underwent ulceration and healed up and the animal did not die of tuberculosis. Koch made a number of experiments which culminated in his attempting the treatment of tuberculosis by the injection of glycerine broth cultures of the bacillus in which all organisms had been killed by heating.

Tuberculin is prepared by growing the bacillus in flasks of a broth containing 4 per cent. of glycerine, at the end of four or six weeks the growth has ceased and the contents of the flasks are collected, evaporated over a water bath to one-tenth the volume, and filtered through a Pasteur-Chamberland filter. The filtrate is crude tuberculin.

As it was found that tubercular individuals developed so great a reaction to the tuberculin, and that the disease was accelerated rather than inhibited, the use of tuberculin as a therapeutic agent has been discontinued, but as a means of diagnosis in cattle and horses is largely adopted.

The suspected animal is injected with the tuberculin, and if there be any latent tuberculosis develops a severe reaction, with rise of temperature, &c. According to Bang the error does not exceed 3·3 per cent. of infected animals inoculated.

---

[1] McFarland's "Pathogenic Bacteria," p. 226.

Antiseptics.—A 5 per cent. solution of carbolic destroys tubercle bacilli in thirty seconds (Yersin). The exposure required in the presence of albuminous material is much longer.

Light.—The tubercle bacillus is destroyed by insolation in a few moments when in a thin layer, and by diffused light in five days (Koch).

Immunisation.—MacFadyean[1] has recently succeeded in producing a considerable degree of immunity in cattle by the repeated injection of tuberculin, followed by the injection of living tubercle bacilli. No anti-tuberculous serum has yet been prepared.

(8) PNEUMOBACILLUS, OR B. FRIEDLÄNDER.

This bacillus, discovered by Friedländer in 1883, in pneumonic sputum, was thought by its discoverer to be the organism causing pneumonia. The *rôle* played by the pneumococcus of Fränkel and Weichelsbaum in pneumonia has, however, been conclusively established, and the B. Friedländer is generally considered as an adventitious bacterium. It often occurs in the lungs in other pathological conditions than croupous pneumonia. It is only slightly pathogenic for animals.

Morphology.—A short bacillus, often so short as to resemble a coccus, generally occurring in chains of four, or in pairs. A distinct capsule is generally present in specimens obtained from the sputum direct, this capsule is similar to that observed in the pneumococcus, and may be stained in the same manner. The capsule often encloses four elements, giving the appearance of a single rod, especially when deeply stained.

Staining Reaction.—Stains with the ordinary aniline dyes, but decolourises when stained by Gram's method.

Biological Characters.—Aerobic, facultative anäerobic, non-motile bacillus. Does not form spores, and does not possess flagella. Gelatin is not liquefied—forms gas and ferments carbohydrates.

Gelatin Stab, 22° C.—White, well marked growth, shining raised convex edge, regular on surface, along the stab well marked growth of white colonies, beaded, the whole described by Friedländer as "nail-shaped." No liquefaction occurs. Bubbles of gas are often found along the line of puncture.

Gelatin Streak, 22° C.—Raised, shining white heavy growth, no

[1] *Trans. Path. Soc.*, Jan., 1902.

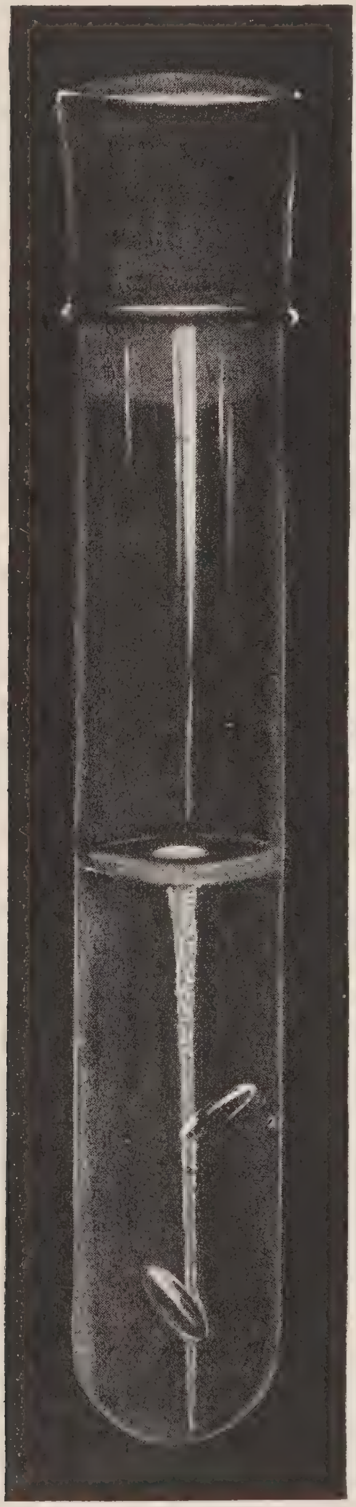

FIG. 43.—BACILLUS FRIEDLÄNDER.

Gelatin stab, showing nail growth and gas bubbles. (From Curtis' "Essentials of Practical Bacteriology.")

liquefaction. Growth rather viscid. Deep, oval or round, entire, brownish opaque. Surface—round convex white.

Gelatin Plates, 22° C.—Microscopically, round, entire, brown or yellow, opaque with transparent borders.

Agar Streak, 35·5° C.—White glistening, heavy, viscous growth, spreading.

Blood Serum, 37·5° C.—Similar to agar, very viscid.

Potato, 22 C.—Well marked, quickly covering the whole surface with viscid, shining whitish-yellow layer, gas bubbles are often seen in the growth.

Broth, 37·5° C.—Turbid, with slimy sediment. Indol slight, H_2S. slight.

Litmus, Milk.—Well marked acid reaction in forty-eight hours, milk clotted in seven to ten days.

Carbohydrates.—Dextrose, maltose, lactose, dextrin, &c., fermented with fermentation of ethyl alcohol, various acids (lactic and acetic) and carbonic acid gas and hydrogen.

Thermal Death Point, 58° C.—(Sternberg). Optimum temperature 37·5° C., but grows at 16° C.

Pathogenesis.—Variable: rabbits are not affected, guinea-pigs slightly. In Friedländer's original experiments, one dog out of five injected, six guinea-pigs out of eleven, and the mice (thirty-two) succumbed to intra-thoracic inoculation, with cultures suspended in distilled water.

(9) BACILLUS INFLUENZÆ (*Pfeiffer's Bacillus*).

This organism occurs in the bronchial secretions and in the blood of persons suffering from influenza. It has so far not been found in other diseased conditions. It is often found in the pneumonic lung of persons dying from that form of influenza, and has quite recently been described as present in certain forms of suppuration following an attack of the disease, as well as in empyæma following influenza. The organism is extremely difficult to cultivate, and rapidly dies out on the culture media used. Canon, who described the organism simultaneously with Pfeiffer, demonstrated its presence in the blood of influenza patients in the following way:—The film is fixed in absolute alcohol for five minutes and then transferred to Czenzynke's stain.

Concentrated aqueous solution of methylene blue	40
0·5 per cent. solution of eosin in 70 per cent. alcohol	20
Distilled Water	40

The films are stained in this solution for three to six hours, and kept in the incubator (hot) during the process, after which they are washed, dried, and mounted in Canada balsam.

The erothrocytes are stained red, leucocytes blue, and the bacilli blue.

The organisms are sometimes found in masses, but as a rule a prolonged search is necessary before they are found, and then often in only small numbers. Pfeiffer was, however, unable to confirm the presence of the organisms in the blood.

Morphology.—Small bacilli 0·5 μ long, by 0·2 μ wide; solitary, or united in pairs, and occasionally in chains of three or four.

Staining Reactions.—The bacilli stain badly with the ordinary stains, best with carbol-fuchsine dilute, or with Lœffler's alkaline methylene blue, or Czenzynke's stain.

Polar staining is generally well marked, the bacilli giving the appearance of diplococci.

They do not stain by Gram's method.

Biological Characters.—An äerobic (facultative anäerobic ?), non-motile bacillus. Not known to form spores. It does not grow upon gelatin.

Glycerine-agar appears to be the only medium upon which the organism grows at all well, and even upon such a medium the development is scanty, and often requires a lens to demonstrate its presence.

The colonies are very small, transparent, and difficult to see.

According to Kitasato, the colonies always remain separate from one another, and do not coalesce as the majority of organisms do. This is considered typical and diagnostic of the organism (Kitasato).

Broth.—A small amount of growth occurs at the surface and gradually sinks, producing a slight woolly deposit.

The bacillus rapidly dies when dried and succumbs to a temperature of 60° (Pfeiffer).

The optimum temperature is that of the body.

Pathogenesis.—Rabbits and guinea-pigs often die when injected intravenously with cultures of the influenza bacillus, the chief symptoms, according to Pfeiffer, being a great rise of temperature, with subsequent paralysis of the hind limbs. Deline and Kole found that they could not produce immunity by the inoculation of gradually increasing doses of the bacillus, as is the case with many other bacteria (*cf.* diphtheria). A certain amount of temporary immunity

resulted in Deline and Kole's experiment, but the animals were never capable of resisting large doses, and no true resistance was developed.

This experimental fact is interesting in relation to the clinical fact of the absence of protection afforded by an attack of the disease, the susceptibility to a second attack being rather increased.

It is probable, however, that after a large number of attacks some slight immunity may be set up, as the disease appears to gradually become less and less severe in type.

(10) B. PYOCYANEUS.

(*Pseudomonas Pyocyanea Migula.*)

This organism is found in abscesses when the contents are of the peculiar bluish or green colour known as "blue pus."

It occurs occasionally in the mouth and throat, and may be found at times in the cavities of tubercular lungs, in otitis media, meningitis, &c. It is not uncommon in dust. On agar cultures especially this organism produces well marked crystals. One of the most characteristic appearances associated with this organism is the green-blue pigment that is formed on most media. Gessard has shown that this pigment is composed of at least two different bodies (fluorescin and pyocyanin), and that by modifying the conditions under which the organism is grown it may be made to produce one or other at will.

Morphology.—Slender bacilli 1·5 to 2 μ in length, and 0·25 to 0·5 μ in thickness; at times two or more may be found jointed together. The rods are actively motile, and possess flagella. No spores are formed.

Staining Reactions.—Stains by the ordinary aniline dyes, and retains the stain of Gram's method.

Biological Characters.—Aerobic, facultative, anäerobic, motile, liquefying bacillus. Forms pigment. No spores formed. Pathogenic for animals.

Gelatin Stab, 22° C.—In twenty-four to thirty-six hours a faint grey line appears along the needle track, and at forty-eight to seventy-two hours liquefaction commences, and a slight green tint appears in the upper part of the gelatin. The liquefaction proceeds in cup-like form, and a green colour at the same time diffuses through the gelatin. The liquefied gelatin is cloudy and shows a deposit in the deeper parts.

Gelatin Streak, 22° C.—A groove of liquefaction is produced in forty-eight hours, the medium becoming gradually tinted green.

Gelatin Plates, 22° C.—Small whitish points appear, which under the microscope are brownish-yellow with nodular surface, and surrounded with a sphere of liquefaction in the deep layers. On the surface the colonies are flat, edge entire, surface reticulated as seen under the microscope, and surrounded with shallow cups of liquefaction. A fine haze of greenish colour appears round each colony, rapidly permeating the whole plate. By about the fifth day the plate is entirely liquefied.

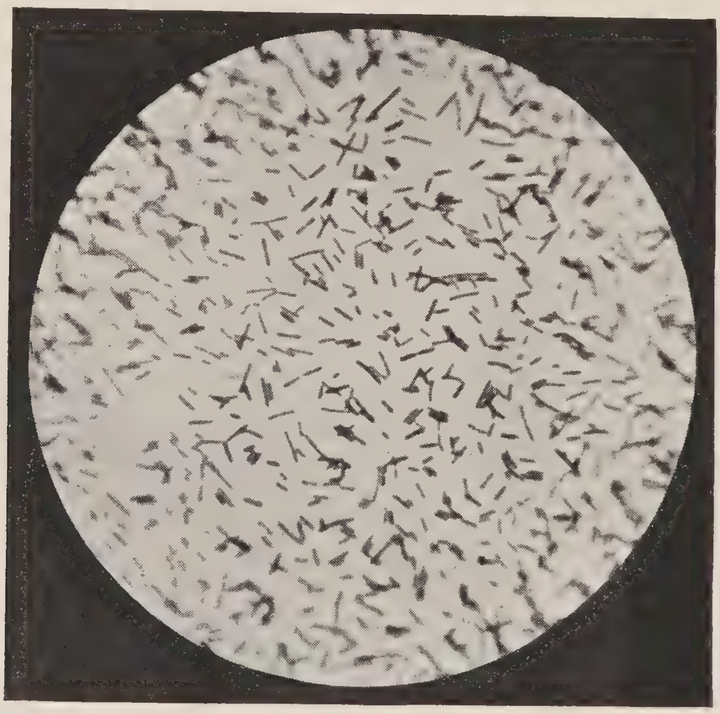

FIG. 44. BACILLUS PYOCYANEUS.
Twenty-four hours' agar cultivation. × 1000.

Agar Streak, 37·5° C.—In twenty-four hours a well-marked moist white layer is formed, and slight tinting of the medium has occurred. In forty-eight hours the colour is bright green due to *fluorescin*, which is soluble and so diffuses through the medium. Later the green colour changes to an olive green or reddish-brown from the development of a second pigment, *pyocyanin*, which requires the presence of peptone for its formation, and is insoluble. Upon fresh agar cultures well marked crystals are seen along the line of growth.

Potato.—Luxuriant growth, at first green, later turning brown, both at 22° and 37·5° C.

Blood Serum, 37·5° C.—Similar to agar, but liquefaction occurs in a well-marked groove.

Litmus Milk, 37·5° C.—Well marked coagulation of casein, which later is dissolved and ammonia given off.

Broth, 37·5° C.—Well marked fluorescence and general turbidity. A scum, often a distinct pellicle, is formed, and a thick precipitate collects at the bottom of the tube. Both pigments are produced; the fluorescin being soluble in chloroform may be separated for the insoluble pyocyanin.

Peptone Water.—With the addition of 5 per cent. glycerine the blue pigment pyocyanin only is formed. No indol formed.

Egg Albumin.—The green pigment fluorescin alone is formed. It is soluble in chloroform, and crystallises out as long needles. On the addition of weak acid the colour changes to red.

Glucose Broth.—Acid, no gas. Nitrates reduced to nitrites.

Pathogenesis.—One cubic centimetre of broth culture injected intraperitoneally or subcutaneously generally causes death in rabbits and guinea-pigs in twenty-four to thirty-six hours. At the autopsy inflammatory œdema and infiltration, sometimes a well-defined abscess, are found at the seat of inoculation. The peritoneal cavity shows a similar fibrinous inflammation when the organism is injected into that cavity. The organisms may be found in small numbers in the blood and various organs.

Intravenous injections generally produce rapidly fatal septicæmia with nephritis, occasionally chronic wasting accompanied with albuminuria. Immunity may be produced by the injection of gradually increasing doses, commencing with a sub-fatal dose. The animals thus immunised show a decidedly increased resistance to infection by the anthrax bacillus. Woodhead and Wood also found that the injection of sterilised cultures of B. pyocyaneus directly following injection with anthrax bacilli protected against that organism.

A large number of varieties of this organism have been described, some of them being no doubt varieties of the B. pyocyaneus, in which the power of pigment production has become, as Gessard has shown it may, so modified that the production of either pigment may be prevented by alteration of the nutrient medium. The antagonism of the B. pyocyaneus and B. anthracis referred to above

is interesting as an example of protection afforded by dissimilar diseases. The relation of the B. pyocyaneus to the tetanus bacillus is of quite another order. The tetanus bacillus, under ordinary circumstances a strict anäerobe, will grow in broth freely exposed to the air if B. pyocyaneus is also present in the culture.

There is usually little difficulty in recognising the B. pyocyaneus, when obtained from pus or other material, by the peculiar pigments formed.

(11) STREPTOTHRIX ACTINOMYCES.

The fungus of actinomycosis was discovered in 1877 by Bollinger, although Langenbeck, as early as 1845, had found that the disease of cattle known as actinomycosis could be transmitted to man.

The disease is almost confined to animals, for the most part cattle, but since attention has been directed to the disease it appears to be more frequent in man than was at first supposed, and a large number of cases are now on record.

The organism itself belongs to the higher bacteria, and shows a far greater complexity of form than is exhibited by the majority of schizomycetes.

The point of infection is commonly the mouth, the organism gaining access to the tissues either through a carious tooth, or as the result of some slight local injury. In a number of cases inoculation has apparently taken place through the medium of an awn of barley containing the fungus, which has become imbedded in the soft tissues. It is common to find these grains imbedded in the local lesion. Two chief varieties of the disease are known, the one in which considerable local reaction with enlargement and thickening of the tissues and bone occurs, the other a condition of general infection with deposits and abscesses in various organs, notably the liver. The chronic form with local swelling was for a long time confounded with osteo-sarcoma. In the pus from the abscesses or local lesion small yellowish-grey granules are to be seen even with the naked eye. Microscopically these granules show the peculiar rosette-shaped fungoid masses, consisting of a central mass surrounded by threads which give the rayed appearance to which the fungus owes its name (Hertz). The granules are each composed of a central mass of cocci-like bodies (gonidia) often containing a quantity of dark pigment. Surrounding the central portion is a zone of tangled threads showing true branching, generally lateral.

The ends of the threads are commonly but not invariably clubbed, and at the periphery of the granule give the appearance of rays. The threads are about 0·5 μ in diameter, and are composed of a central protoplasmic axis surrounded by a gelatinous sheath. In young specimens the threads take up aniline dyes uniformly, but in old cultures the threads tend to stain irregularly and may appear as chains of cocci or bacilli. The clubs do not stain by Gram. In culture media the changes are somewhat different. The branched and tangled mass of threads are formed as colonies of cartilaginous consistency; the clubbing is not marked. The threads stain irregularly after about a week, and the gonidia are well marked, often covering the colony with a white or yellow dusty efflorescence. The typical granules are not formed, although there may be some attempt on liquid blood serum.

The conditions of growth outside the body and the form in which the organism exists when infection occurs is not at present known. It is thought that it may exist in the grains of certain cereals in a similar manner to Puccinia graminis.

Morphology.—Filamentous, branched, and club-shaped forms (fig. 1, *i.*), with all the morphological forms of bacteria represented at times. The clubs are not formed in cultures. Various changes occur in the threads, which at first stain well but later become granular and stain irregularly. No endospores are formed, but gonidia are present.

Staining Reactions.—Stains with the ordinary aniline dyes, best by Gram's method, which is much the best stain for tissue preparations. The clubs do not retain the stain of Gram's method, but may be counterstained with picric acid.

Biological Characters.—An äerobic facultative anäerobic streptothrix, forming gonidia, non-motile; does not possess flagella. Gelatin is liquefied.

Gelatin.—The organism grows slowly at the room temperature, and the medium is gradually liquefied and turns a dark brown colour, the liquid being somewhat viscous. Scattered about in the fluid are small round white nodules, from which filaments radiate.

Agar and Glycerine Agar.—After three days at 37·5° C., minute, hard, spherical, white colonies appear (fig. 45, A); these gradually increase, and become raised at their edges, ultimately forming an undulating and crater-form surface, at first yellowish, later greenish-grey (fig. 45, B). The older colonies often resemble lichen (fig. 45, C),

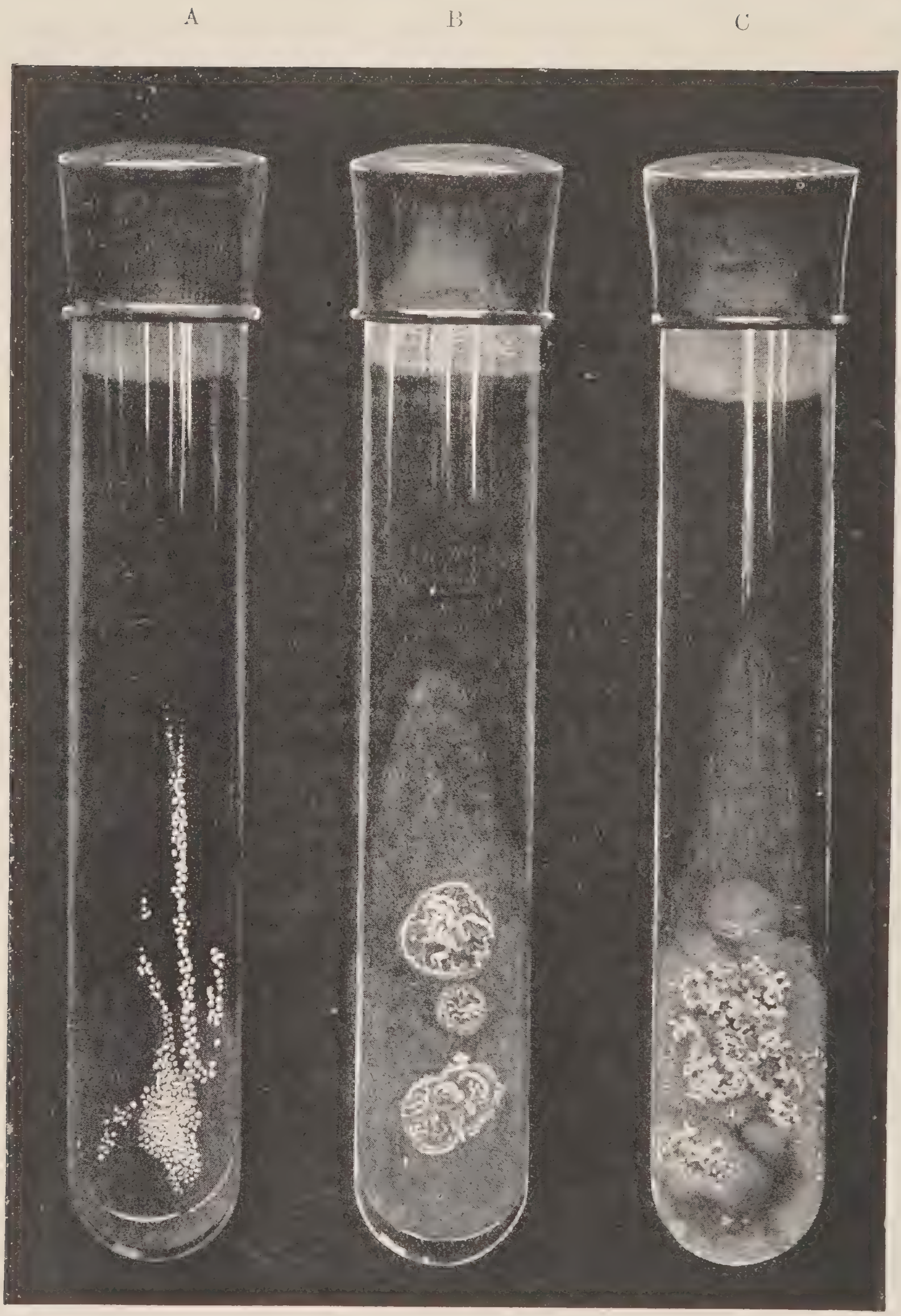

FIG. 45.—STREPTOTHRIX ACTINOMYCES CULTIVATIONS ON GLYCERINE AGAR.

A. Discrete rounded colonies, after about ten days' incubation at 37° C. B. Limpet-shaped colonies three and a half months old. C. Lichen-like appearance frequently seen; the growth is three and a half months old. (From Curtis' "Essentials of Practical Bacteriology.")

and have a yellowish or ashen-grey tint. The corrugated surface is covered with a powdery dusty layer. The colonies are extremely difficult to remove for examination.

Potato.—Similar appearance to agar, but more luxuriant growth. The colonies are quite unlike those produced by other bacteria, the streptothrix of madura-foot being the only organism at all resembling them, and this organism colours the potato a dark red.

Pathogenesis.—Intra-peritoneal injections of the bacillary or filamentous form of the parasite in rabbits and guinea-pigs is followed in about a month by nodule formation. The nodules, composed of granulation tissue (granuloma), are vascular on the surface, and contain curdy pus, in which the typical colonies are found. In man the disease may take one or both of the forms noted above. Sometimes large areas of bone become carious and necrosed, the disease being classed by Virchow with glanders and tubercle as infective granulomata. Infection of the bowel may occur, ulceration and extensive necrosis following. The organism has also been described in the ovaries and fallopian tubes (Muir and Granger Stewart); it has also been found in the brain, liver, spleen, &c. In the later stages of the disease deposits may occur in the various organs with the formation of metastatic abscesses containing the typical colonies. The diagnosis is easy, both by the typical granules in the pus of the abscesses or other lesion, and the characteristic growth on agar and potato.

(12) BACILLUS GINGIVÆ PYOGENES (Miller).

Found by Miller in unhealthy mouths and along the gum margin in such cases. I have also observed this organism in several cases both of dental caries and in gingival inflammation, and have therefore worked out the biological characters, as those given by Miller only include growth on gelatin and agar.

Morphology.— Bacilli from 2 to 6 μ long, 0·5 to 0·75 μ wide, often jointed in pairs or in chains. The elements may at times be curved. Ends square or rounded. Two or three bacilli may at times lie side by side somewhat in the manner of the Klebs-Lœffler bacilli. Involution forms (globular or twisted) are common on old cultures.

Staining Reactions.—Stains by the ordinary aniline dyes and by Gram's method. The flagella may be stained by Pitfield's method.

Biological Characters.—An äerobic facultative anäerobic, liquefy-

ing, motile, chromogenic bacillus. Forms spores which resist a temperature of 75° C. for half an hour. Grows in the usual culture media, best at 37·5° C.

Gelatin Plates, 22° C.—In forty-eight hours irregular spreading, raised colonies, with irregular and wavy edge, yellow centre lying above the outer paler mass of colony. In three days the colonies liquefy the gelatin and float upon the surface of liquid as round crinkled masses with thickened centre, which is now yellow-brown in colour. The gelatin becomes dark brown.

Gelatin Stab, 22° C.—Forty-eight hours, growth to bottom of stab; three days, slight cup-shaped liquefaction which gradually approaches the walls of the tube. In newly isolated cultures the cone may remain empty. White flocculi form in the fluid later.

Gelatin Shake, 22° C.—Forty-eight hours, cloud of minute colonies but no gas bubbles. Liquefaction commences at the surface, and does not take place in the depths.

Gelatin Streak, 22° C.—Forty-eight hours, slight liquefied groove with little other evidence of growth; four days, the fluid becomes filled with yellowish-white flocculi with radiating processes.

Agar, 37·5° C.—Twenty-four hours, heavy growth, with tendency to spread at intervals along the streak in club-shaped processes (lobulate); the central portion of the streak and of the club-shaped processes is buff-yellow, the edge grey-white. The medium may be tinted brown.

Blood Serum, 37·5° C.—In twenty-four hours a broad, deep groove of liquefaction is formed with brown discoloration of medium.

Potato, 37·5° C.—Twenty-four hours, well-marked, dry, yellow-brown growth, granular and glistening; the potato becomes coloured throughout.

At 22° C. a good deal of yellow pigment is formed.

Broth, 37·5° C.—Twenty-four hours, faint growth of isolated flocculi in fluid; no general turbidity. The flocculi sink and form a thin deposit in three or four days. Good indol reaction in seven days. H_2S. scanty.

Litmus Milk, 37·5° C.—Forty-eight hours, well-marked acidity with coagulation which does not become dissolved for two or three days. No gas is formed, and no smell given off.

Glucose broth, lactose broth, maltose broth—acid fermentation in forty-eight hours.

Seven days' agar culture suspended in broth and heated to

75° C. for half an hour gives a good culture on sub-cultivations being made.

The spores, which are small, stain by Möllers' method.

Pathogenesis (Miller).—Pathogenic for mice, rabbits, and guinea-pigs when intravenously injected in doses of 0·25 cc. of a broth culture. At the autopsy peritonitis, sometimes purulent, is observed. Death occurs in ten to twenty-four hours. Subcutaneous inoculation resulted in local abscess only.

(13) B. GANGRÆNÆ PULPÆ (Arkövy).

B. mesentericus niger (*fuscus*).

Found by Arkövy in dead tooth pulps, in carious dentine, and in the oral secretions.

Morphology.—Bacilli about 4 μ in length, with rounded ends (not sharply defined as first stated, and not pleomorphic); often pairs are united at an acute angle.

Staining Reaction.—Stains with the ordinary aniline dyes, and by Gram's method. The so-called cocci described by Arkövy do not stain by methylene blue, and are spores[1] which are large and oval, and may be stained by the usual methods (hot carbol-fuchsin, &c.).

Biological Characters.—An äerobic, facultative anäerobic, motile liquefying bacillus; forms pigment and spores.

Gelatin Plates.—In twenty-four hours minute white colonies make their appearance, resembling flour dust. These gradually become slightly yellow in colour, and in thirty hours are confluent, whilst the whole of the medium is liquefied with a whitish wrinkled pellicle covering and floating on the surface of the fluid. An extremely unpleasant smell is given off resembling old cheese.

Gelatin Stab.—At the end of forty-eight hours liquefaction commences, and soon reaches the wall of the tube. Flocculi form in the fluid, and in ten days a wrinkled pellicle has formed upon the surface. The gelatin is coloured a red brown, the pellicle being of a dirty brown. The liquefied gelatin gives a strongly alkaline reaction.

Agar Plates, 37·5° C.—At the end of twenty-four or thirty-six hours, small white colonies make their appearance. They have a

[1] *Cent. für Bakteriol.*, Bd. xxix., No. 19, 1901.

flour-dust form similar to those on the gelatin plates. Occasionally the colonies (surface ?) are larger, flat and leaf-like, and marked with fine striæ. The same unpleasant smell noticed in the gelatin plates is also present.

Agar Streak, 37·5° C.—A wrinkled layer, five to six millimetres broad, is formed, which becomes brown after five or six days; the medium is itself also coloured a brownish tint.

According to Rader's international colour scale, the tints produced on the different media are as follows:—

(1) Agar, colour of growth on surface ...	33 brown—*a*
(2) Gelatin reflected light	33 brown—*d*
(3) Gelatin transmitted light	cinnabar—3*a*

Blood Serum, 37·5° C.—A brown liquefied streak is produced along the needle track.

Broth, 37·5° C.—In thirty-six to forty-eight hours a well-marked pellicle is formed, having the same colour as the gelatin cultivation.

Potato, 37·5° C.—A moist brownish wrinkled skin forms over the surface, and the medium is coloured a deep brown.

Milk, 37·5° C.—No acid is formed, but a precipitation of the casein occurs.

Pathogenesis.—Subcutaneous inoculation in mice produced diarrhœa and death in twelve days. The bacilli were found in the blood. The bacillus is also pathogenic for rabbits and guinea-pigs when injected in large doses subcutaneously.

(14) BACILLUS DENTALIS VIRIDANS (Miller).

Found by Miller in the superficial layers of carious dentine.

Morphology.—Slightly curved bacilli with pointed ends, solitary or in pairs.

Biological Characters.—An äerobic, facultative anäerobic, non-liquefying bacillus. Spore formation, motility, staining reactions not given.

Grows well in the usual culture media.

Gelatin Plates.—Spherical colonies with concentric rings, almost colourless except under the microscope, when they are slightly yellow. The gelatin is coloured a faint green.

Gelatin Stab.—A limited growth occurs along the track of the needle and a considerable growth upon the surface. No liquefaction occurs.

Agar Streak.—A thin growth with an irregular margin occurs along the track of the needle. The growth is bluish by transmitted light and greenish-grey by reflected light.

No other cultural characters given.

Pathogenesis.—Injections into the peritoneal cavity of mice and guinea-pigs usually cause fatal peritonitis in from one to six days; the bacilli are found in the blood in small numbers. Subcutaneous inoculation into animals produced severe local suppuration.

(15) BACILLUS PULPÆ PYOGENES (Miller).

Obtained by Miller from a gangrenous tooth pulp.

Morphology.—Bacilli slightly curved and pointed, occurring in chains, in pairs, or solitary.

Biological Characters.—An äerobic, facultative anäerobic, liquefying bacillus. Spore formation, motility, staining reactions not given.

Gelatin Plates.—Large, darkish, yellow-brown colonies appear, which in eighteen hours produce liquefaction, and soon liquefy the whole of the gelatin.

Gelatin Stab.—Liquefaction begins within forty-eight hours and gradually extends, the liquefied gelatin being separated from the non-liquefied portion by a horizontal plane.

No other biological characters given.

Pathogenesis.—Injections of 0·05 cc. into the peritoneal cavity produced death of white mice in eighteen to thirty hours.

(16) MICROCOCCUS GINGIVÆ PYOGENES (Miller).

Obtained by Miller from a case of suppurative periodontitis three times at intervals of three months, and once in a very dirty mouth.

Morphology.—Irregular cocci or plump rods, occurring solitary or in pairs.

Biological Characters.—An äerobic, facultative anäerobic, non-liquefying micrococcus. Grows well at the room temperature and in the usual culture media.

Gelatin Plates.—Forms spherical, well-defined colonies, with a sharp margin, which at first are colourless under the microscope, but later become opaque.

Gelatin Stab.—A well-marked growth occurs along the line of puncture and a copious raised growth on the surface. No liquefaction occurs.

Agar Streak.—A thick, greyish, moist growth occurs in twenty-four hours, which has a purple tinge by transmitted light.

Sugar Media (composition not stated).—A considerable development of gas occurs and a strongly acid reaction is soon present.

Pathogenesis.—Subcutaneous injections into mice were followed by local abscess and necrosis, and sometimes by the death of the animal. Intraperitoneal injection invariably produced death in twelve to twenty-four hours.

[Staining reactions and growth on milk, potato, broth, &c., not given. This organism is probably nearly related to B. coli communis.]

CHAPTER VIII.

Dental Caries.

The destruction of the tissues of the teeth, commonly known as dental caries, is a process distinctly allied, both in its chemical and bacteriological aspects, to the general phenomena of putrefaction.

Dental caries, like putrefaction, is rarely caused by one species of organism, and the reaction of the medium in both caries and putrefaction undergoes fluctuation from acid to alkali; and in both processes an alternation of species takes place according to the phase of phenomenon.

The disintegration of enamel, dentine, and cementum is brought about in the first instance by the action of various organic acids, mainly lactic, produced by the vital activity of bacteria; subsequently the various digestive ferments also produced by the organisms come into operation, dissolving the decalcified matrix of the cementum and dentine. What, however, occurs in the first stages of dental caries, and why certain races of men and the majority of animals appear to be immune, is a much more difficult matter to elucidate.

Artificial caries, so well demonstrated by Miller, is by no means difficult to reproduce by exposing cubes of dentine to the action of micro-organisms of the acid-producing class in solutions containing carbohydrates.

Caries of enamel is much more difficult to reproduce artificially, uniform denudation of the enamel generally taking place in carbohydrate cultivations; and as the process of natural caries generally originates at some point of the enamel surface rather than over the whole tooth, much interest centres around the initiation of the process.

Very many theories have been advanced at various times to explain the commencement of enamel destruction, ill-developed and pathological conditions of the enamel structure, deficiency of

lime salts—said to be associated with insufficient calcium salts in the water of a country—diet, civilisation, alteration of physiological relationship in position of the teeth in the jaw from evolutionary causes, &c. Many, if not all, of these factors may have their place in predisposing causes, but the ultimate liberating cause is often overlooked in the multiplication of predisposing ones.

Read[1] has lately advanced the proposition that caries may be due to the alteration in the sort of flour used in bread-making. Thus the finer roller-ground flour has to a large extent replaced the old stone-milled article; and Read states that the amount of acidity in terms of lactic acid produced by chewing bread made from either sample is much greater in the case of the finer roller flour.

Samples of bread made from the two flours were chewed for equal lengths of time and the acidity determined; in every case the acidity of the roller flour was in excess of the stone-milled. That such an acidity might predispose to caries is by no means improbable, but the subject is not yet sufficiently investigated to draw definite conclusions.

Sim Wallace[2] considers that "the cause of the prevalence of dental caries is that the natural food-stuffs are to a large extent ridded of their accompanying fibrous parts," and that due to the same cause "the micro-organisms of the mouth lodge and multiply, and augment the rapidity and intensity of the acid fermentation."

That such a factor does occur is certain, but it hardly explains the fact that animals, dogs, cats, monkeys, rabbits, &c., which exhibit no dental caries, are yet found to have food particles frequently lodged between their teeth after a meal, the fibrous matters themselves remaining impacted. Interstitial caries is rare in animals and yet commonly occurs in man.

The researches of Miller, Mummery, and more recently Leon Williams, have shown that the enamel surface in sheltered positions is often covered with a film-like layer of bacteria, and that under such a layer definite disintegration of the enamel is taking place. Moreover many of the bacteria found in this film may be seen in the spaces hollowed out between the enamel prisms; the spaces evidently have been formed by the action of the acids produced by the bacteria; moreover spreading from these points of attack

---

[1] *Journ. Brit. Dent. Assoc.*, 1900. [2] "Cause of Decay in Teeth."

and passing inwards to the dentine may be seen organisms well in advance of the general process.

The acid produced by the bacteria, although in small quantities, would eventually inhibit the growth of the organisms were it not neutralised as soon as it is formed by union with the lime salts of the enamel, each fresh increment of acid attacking a fresh portion of tissue, the resulting products diffusing into the saliva.

Miller figures several examples of this bacterial layer attached to the surface of enamel, and also shows the organisms permeating the

FIG. 46.—DENTAL CARIES. SECTION OF ENAMEL WITH LAYER OF ATTACHED ORGANISMS AND FORMATION OF PITS BETWEEN ENAMEL RODS. Photomicrograph and specimen by Dr. Leon Williams. × 500.

enamel substratum. Miller also showed, by a series of admirably conducted experiments, that the familiar appearance of caries is due to the various acids evolved from the carbohydrate constituents of a normal diet.

Leber and Rottenstein endeavoured to produce artificial caries by placing normal teeth in a mixture composed of the various constituents of a normal diet. They failed to produce caries experimentally, and came to the conclusion that it was not the result of bacterial activity. They admitted that their experimental

flasks, &c., gave off obnoxious smells, and that ordinary putrefaction occurred, in fact they abandoned their experiments largely on this account. Miller however by so adjusting the condition that a constant acid reaction was present, obtained strikingly confirmatory results of the *rôle* played by organisms producing acid fermentation in the pathological condition known as dental caries.

Leber and Rottenstein's failure is of easy explanation, but although they failed in their main object their work is of considerable importance in the ætiology of caries as demonstrating by a negative result the necessity of an acid fermentation to initiate the process.

In the ordinary phenomenon of putrefaction an acid reaction may be present, but very soon gives place to distinct alkalinity and the evolution of evil-smelling gases—indol, skatol, sulphuretted hydrogen, and, as a rule, ammonia.

The phenomena of putrefaction are by no means all produced by a single species or race of organisms, one succeeding another as the conditions suitable for their development arise (see p. 21).

But more important still is the observation of Maly,[1] who took the mucous membrane of the stomach, placed it in a solution of cane sugar and kept the mixture at the body temperature for several days. The lactic acid arising from the decomposition of the sugar was neutralised from time to time, and it was found that the process of acid production continued until all the carbohydrate present had been converted into lactate; and then, and not till then, did putrefactive odours become manifest.

This, then, fully explains the failure of Leber and Rottenstein, and the success of Miller's experiments.

It will be noticed, moreover, that the acid produced by the organisms requires to be neutralised from time to time; otherwise, owing to the increased acidity of the fluid, the organisms cease their activity, the very acid produced acting as a check to the growth of the bacteria producing it.

In examining the statistics of the incidence of caries as occurring in various races of mankind, and in considering the diet of such people, we find that those races whose diet is mainly meat show a far smaller percentage of caries than do the races whose diet is principally carbohydrate.

---

[1] *Herman's Handbuch*, Bd. v. (2), S. 239.

It is moreover observed that the teeth of persons suffering from enteric and other diseases necessitating a prolonged subsistence, during convalescence, on a diet composed of a considerable proportion of carbohydrate, often show an increased amount of caries unless most careful cleansing of the mouth is carried out by the attendants. Millers, bakers, and persons engaged in sweet-stuff factories invariably exhibit a marked amount of caries; in millers and bakers particularly, marginal cavities are common. Caries is moreover more common in children of both sexes than in adults, and more frequent in adult woman than in man. All the various questions relating to fermentation point to food-stuff composition as of great importance in the ætiology of dental caries, but in applying this general principle we are met with several obstacles which apparently militate a good deal against the acceptance of the theory of food-stuff origin of caries.

One of the chief and main difficulties in discussing the whole problem is the attempt to explain the process of dental caries without having regard to the importance of several rather than a single predisposing cause; the explanations advanced by various observers are none of them sufficient *per se* to elucidate the problem in its entirety, but taken together a very good working hypothesis may be obtained. Dental caries is not a specific disease due to a certain specific micro-organism; it is no definite "entity," but a process occurring through the operation of certain biological and physical laws.

It has been suggested by some observers that a deficiency in the amount of lime salts of teeth may contribute to the early development of dental caries.

Black, and later C. Tomes, estimated the percentage composition of a large number of teeth and found that there was no appreciable variation, and that there was no evidence that the incidence of caries could be associated with a decrease in the percentage of the lime salts present.

It is known however from an empirical point of view that some teeth are apparently mcre liable to the inroads of bacteria than are others, although the researches just cited show that the susceptibility to caries probably does not lie in the lime salt content.

Leon Williams has demonstrated the plaques of micro-organisms on the enamel surface of teeth, and the incipient caries occurring under these plaques, and I have myself constantly observed these

sheets of bacteria in various situations on the enamel surface. During investigations carried on concerning the flora of carious teeth I have constantly met with a series of bacteria which are characterised by a curious facility for forming extremely tough gelatinous colonies, not by any means due to the presence of carbohydrate as in the mucinous fermentation of sugar and molasses, but occurring on media free from any carbohydrate whatever; one organism in particular, a coccus, is frequently present. This organism is frequently to be met with upon the enamel surface of teeth, particularly the white opaque patches of softened enamel to which Williams has drawn attention (for description see p. 172).

I found no difficulty in reproducing the plaque-like layer upon sterilised teeth suspended in a cultivation of one of these bacteria, and moreover when another organism capable of acid fermentation was mixed with the plaque-forming organism, and carbohydrate media used, under the plaques formed upon the enamel surface by the two bacteria superficial disintegration of the enamel was observed to occur in a week to ten days. Such an experiment is no doubt largely in favour of the organisms; there is no cleansing due to mastication or movement of tongue or saliva, and no great and constant dilution of the acids formed, which are at liberty to attack the tooth under the bacterial sheet. Nevertheless the colonies, which are often formed even upon the surface of the glass in the culture tube, are remarkably adherent and resist removal, and it is not unfair to suppose that such a condition obtains in the mouth. An important coincidence to this supposition is afforded by the fine teeth of many native races, many of which, particularly the Zulus and Kaffirs, are particularly assiduous in cleansing their teeth. Amongst the former it is the common practice for the Zulu mother to carefully cleanse her child's mouth after every meal until it is old enough to do so for itself; the finger is generally used, and some ashes (wood) from the fire are employed. The majority of the adult natives in the beds of the Seamen's Hospitals are especially careful of their teeth, at times refusing to eat unless first supplied with water with which to wash their mouths after the meal.

In making cultivations from the mouths of natives with good dentition, and also from the mouths of some of the monkeys at the Zoological Gardens, I have been struck with the number of putrefactive rather than acid-forming bacteria present in the mouths.

Certain of these bacteria will also form a definite layer upon the surface of enamel when a sterilised tooth is suspended in a broth culture.

It is a frequently observed clinical fact that individuals applying for treatment at dental hospitals may possess peculiarly dirty mouths, with marginal inflammation of the gums, and yet exhibit extremely small evidence of caries; the appearance of such mouths is strikingly similar to several of the monkeys I examined. Putrefaction was evidently the ascendant process and therefore an accompanying alkaline reaction, such carbohydrate food as was taken probably happening to be of a species only fermented with difficulty. The coincidence is interesting, and if open to several explanations is not opposed to the general principles we have discussed.

I have already referred to the question of roller-flour, and to the increased amount of acid it is said to engender. Acid may be present in the mouth in certain pathological and physiological conditions, in pyrosis, the vomiting of pregnancy; in diabetes mellitus an acid saliva is frequently present. Acid contained in medicine has also to be mentioned in the same category.

It is possible that small quantities of acid frequently applied to the teeth may produce microscopical irregularities, or what is more important, solution of the interprismatic substance of the enamel prisms, or of the axial portions of those prisms, assisting in the adherence of organisms and forming microscopical points of entrance from which portals the process may extend.

On the other hand we must not forget that some people are in the habit of consuming acid foods, and eating strongly acid fruits, as is the case with the Sicilians[1], and it is conceivable that the acid may act as a protective by preventing the development of acid-forming organisms, or dissolving away the outer layers of the enamel and with it the contained bacteria, secondary dentine occluding the pulp chamber before the process had threatened that cavity. Such an obliteration is common in old skulls with great denudation of the dentine.

So far we have not considered the relation of pathological malformations of the teeth in their relation to caries.

---

[1] *Cosmos*, 1898. Dr. Leon Williams tells me that the Sicilians, who are particularly free from caries, are large consumers of lemons.

Leon Williams has pointed out that pits, grooves and fissures, pigmentation, granular and amorphous enamel are to be found in the lower animals whose teeth are comparatively free from caries, and that various species of human enamel, which are apparently especially liable to caries by reason of their pathological irregularities, may resist for years, whereas enamel, to all appearances sound, is often the seat of rapid decay. To such a statement all will agree, but it is an undoubted clinical fact that hypoplasic teeth do certainly undergo extremely rapid caries under certain conditions, the point of attack being almost invariably a pit or fissure on the enamel surface. Looking at the question from a broad general point of view it certainly by no means follows that because a tooth has a developmental defect *therefore it must* become carious, but we may say with a considerable measure of truth that exposed to those conditions which we have seen predispose to caries, the tooth with irregularities and deficiencies on its enamel surface is more liable to attack than the developmentally perfect one. Further, we are not sufficiently conversant with the microscopical defects or normal structure of the enamel surface of teeth generally to disregard microscopical defects as predisposing causes. Moreover the normal fissures of molar and bicuspid teeth are so frequently the starting points of caries that it is impossible to ignore the great importance of points of least resistance. Dental caries, although not a true disease, occurs in a cavity of the body bathed with secretions physiologically unstable, and containing living cells, all subject to various oscillations between disease and health, and we must therefore adopt in the study of caries many of the methods applicable to the ætiology of disease in general. We have seen that the organisms of diphtheria and pneumonia may exist in normal individuals' mouths with no manifestation of disease, that the liberating cause of pathological energy, to wit the bacterium, is less than the resistance of the body cells, and that it is only in a limited number of instances that the balance appears depressed in the favour of the bacterium. A little careful consideration will show that dental caries has many points of similarity, for in some mouths no caries exists, notwithstanding the luxuriant flora present, whilst in others apparently similar in all respects caries is rampant.

Now the teeth themselves cannot be possessed of bactericidal action, whereas the fluids of the mouth are daily undergoing physiological variation, and it seems feasible that alterations in composi-

tion, reaction or quantity of the buccal fluids may furnish many explanatory points in the ætiology of tooth decay. Bacteria are notoriously sensitive to their environment, slight changes of medium, temperature, alkalinity and what not, favouring the development of one species to the exclusion of others; we may therefore briefly conclude that any circumstance or series of circumstances that favours the development of acid-forming bacteria and their adhesion and retention about the teeth, may be rightly considered as a predisposing cause of dental caries.

Food Stuff Chemistry.—Fermentation is of such supreme importance in dental caries that it will be as well to briefly mention some special points relating to fermentative changes.

Food stuffs are divisible into proteids, carbohydrates, and fats; of these carbohydrates are of the chief importance in caries.

Carbohydrates are classed in three main groups according to their chemical composition:—

(1) Monosaccharides.

(2) Disaccharides.

(3) Polysaccharides.

Many other carbohydrates exist, but they are physiologically unimportant.

(1) Monosaccharides ($C_6H_{12}O_6$).—Dextrose, levulose (glucose). These carbohydrates are commonly found in nature, generally together, in fruits, seeds, roots and honey. Galactose, another carbohydrate of the group, is formed from the hydrolysis of lactose or milk sugar.

They are directly fermentable by yeast into alcohol and carbonic acid,

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} + \text{yeast} + \underset{\text{Alcohol.}}{2\,C_2H_5\,.\,OH} + 2\,CO_2,$$

or by several of the schizomycetes of the mouth into lactic acid.

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} = \underset{\text{Lactic acid.}}{2\,CH_3\,.\,CH\,.\,OH\,.\,COOH.}$$

This equation does not exactly express the entire change, as a certain amount of the sugar is used up by the growth of the organisms. To obtain the acid in pure form a fermentation is carried on in a large flask containing sugar (lactose or dextrose) with a layer of precipitated chalk at the bottom. As the acid is produced it combines with the calcium, forming calcium lactate. The lactate is filtered off when the action has ceased, and the acid recovered by distillation with sulphuric acid.

Disaccharides ($C_{12}H_{22}O_{11}$).—Cane sugar, milk sugar (lactose), malt sugar (maltose).

The disaccharides are regarded as condensation products of the monosaccharides with the elimination of a molecule of water.

$$\underset{\text{Dextrose and Lævulose.}}{C_6H_{12}O_6 + C_6H_{12}O_6} = \underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + H_2O.$$

The importance of this is seen in the fact that before fermentation of the higher sugars occurs they require hydrolising to the lower or monosaccharide form.

Cane sugar is not directly fermentable by yeast, but an invert ferment produced by the yeast changes the cane sugar to dextrose and lævulose, which is then fermentable. Some organisms occurring in the mouth are able to transform the sugar direct, but as a rule cane sugar takes much longer to ferment than the glucoses (dextrose and lævulose).

$$C_{12}H_{22}O_{11} + H_2O = C_6\overset{+}{H}_{12}O_6 + C_6\overset{-}{H}_{12}O_6,$$

Or

$$C_{12}H_{22}O_{11} + H_2O = 4\,(CH_3 \,.\, CH \,.\, OH \,.\, COOH).$$

In the experiment, directly inversion of the cane sugar occurs, the solution which before produced no reduction of Fehling's solution now gives a marked reaction.

Maltose ferments readily with yeast and with the majority of mouth bacteria. It forms a typical osazone with phenylhydrazine. It is the chief sugar formed by the action of ptyalin upon starch in the mouth.

Lactose occurs only in milk. It is the most resistant sugar to the effects of yeast, but is fermentable by mouth bacteria, with the formation of lactic acid.

The genus B. lactis, first described by Lord Lister, is composed of a large number of different species. Organisms belonging to this class are invariably present in milk, and may be generally obtained from sour milk by cultural methods.

Polysaccharides ($C_6H_{10}O_5$)n.—A large group of naturally occurring carbohydrates, the chief groups being the *starch* group, the *cellulose*, the *gum* group (dextrines, plant gums and mucilages).

Starch.—Not directly fermentable by yeast; is fermented by a few bacteria occurring in the mouth and intestine. Starch is also inverted to maltose by the action of the ptyalin of saliva, and by ferments produced and contained in certain bacteria, some of which are found in the mouth.

The chemical changes involved are extremely complicated, but the final conversion into maltose may be represented thus :—

$$\underset{\text{Starch.}}{2\,C_6H_{10}O_5} + H_2O = \underset{\text{Maltose.}}{C_{12}H_{22}O_{11}};$$

the fermentation to lactic acid occurring as given above.

$$C_{12}H_{22}O_{11} + H_2O = 4\,CH_3CH\,.\,OH\,.\,COOH.$$

Under certain circumstances butyric acid may be formed, but the quantity is very small. It is generally produced by anäerobic bacteria, and may be formed by a direct change of lactic acid :—

$$2\,C_3H_6O_3 = C_4H_8O_2 + 2\,CO_2 + 2\,H_2.$$

The other sub-groups of the polysaccharides are unimportant.

Proteids.—The proteids undergo fermentation by the action of bacteria with the production of certain alkaloidal substances, which in the presence of air undergo further decomposition. In the absence of free oxygen the later changes do not occur, and the poisonous alkaloids may become absorbed (Hueppe).

The digestive effects of bacteria upon proteids are similar to that of the pancreatic ferment, and traces of organic acids (paraoxphenyl propionic, &c.) may be formed. They have little importance in dental caries.

Fats.—A certain amount of digestion of fat is produced by bacteria with the formation of fatty acids.

The products are unimportant in dental caries as far as it is at present known, and at any rate the amount produced by fermentation of fat in the mouth must be exceedingly small in quantity.

The caries of enamel and the caries of dentine present certain fundamental differences in their pathology coincident upon their different structure, and the mere traces of organic matter contained in the former preclude many of the phenomena which may be observed in the latter.

We have already seen that caries is allied in many respects to putrefaction, and may in fact be considered as a special case, and that the organisms concerned in such a disintegration of tooth tissue are by no means of one species and do not necessarily exist in pure culture in the decomposing tissues. We have also seen that all organisms do not thrive in saliva, and that food stuffs may greatly influence the flora at any time present in the oral cavity ; it follows then that a large number of bacteria of varying species are to be found in decaying dentine, but certain organisms occur more fre-

quently than others, and we are therefore justified in adopting a general grouping of the forms more commonly met with. Some of these are members of well-known species, others have so far only been described as occurring in the mouth.

The bacteria are more numerous upon the surface and superficial layers of decaying dentine than in the deeper layers, while enamel holds an intermediate position. Miller first pointed out, and I have since confirmed his observations experimentally, that the bacteria of tooth decay produce their effects in two ways; firstly, by the production of acids which attack the lime salts of the tooth, and secondly, by the development of proteolytic enzymes which

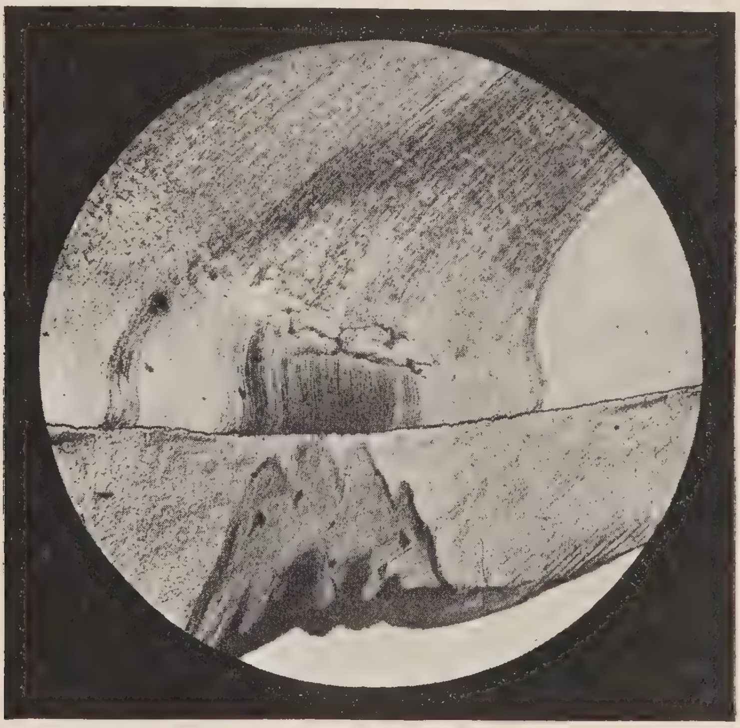

Fig. 47.—Dental Caries affecting Dentine under Enamel Pit; the minute darkly-stained Areas show the Masses of Bacteria.
Photomicrograph and specimen by Dr. Leon Williams. × 250.

digest the matrix denuded of its lime salt, and as a general rule the superficial layers contain a preponderance of the liquefying species, the deep layers mostly acid-forming bacteria. We are therefore able to divide the bacteria of dental caries into two main classes corresponding to the predominant biological function: (*a*) acid production; (*b*) liquefaction.

In enamel decay the liquefying organisms are unable to function

unless they are at the same time producers of acid; in caries of cement both liquefaction and acid destruction may take place together.

Caries of Dentine.—The bacteria concerned in the process of caries in dentine appear to be greatly influenced by their surroundings, inasmuch as those isolated from the deep layers are generally capable of growing under anäerobic conditions—in fact grow better in the absence of free oxygen—whereas the surface and superficial layers are inhabited by organisms that prefer free oxygen. In making cultivations from the deep layers considerable care must be exercised to exclude contamination from the surface. The external surface of a freshly extracted carious tooth should be well seared with a hot iron and the superficial portion of decayed dentine cut away with a sterile excavator or knife; the surface is seared a second time and a second slice of dentine removed with a sterile knife. The lower layers can now be removed with another sterile instrument, ground up in nutrient broth, and cultivations and coverslip preparations made from the emulsion. The organisms obtained in cultures by this method all appear to be rapid acid producers in the presence of carbohydrate food. Arkövy has recently suggested that caries may occur in an alkaline medium, and suggests the introduction of a third class of "*alkali-producers*." I do not think there is much to commend the adoption of such a division as it appears highly improbable that any direct liquefaction of hard undecalcified dentine ever occurs through the agency of proteolytic enzymes of bacterial origin. Arkövy's[1] theory is based on extremely slender evidence, without controls and without any proof that acid reaction was or was not present in the early stages of the process. In all the experiments I have made no liquefaction of hard undecalcified dentine was accomplished, although decalcified dentine was digested by many bacteria obtained from carious dentine. That liquefied gelatin is invariably strongly alkaline is a common fact of laboratory knowledge, most organisms will not liquefy acid gelatin in contradistinction to the liquefaction or digestion brought about by most ferments of animal origin, which require an acid medium. The alkaline reaction of cultures is generally due to ammonia, which has no appreciable action upon the tooth salt. The alkali-producers of Arkövy are really liquefying organisms, and there is certainly no

[1] *Vierteljahrschrift für Zahnheilkunde*, xiv., Heft 3.

occasion to schedule them twice over on account of their alkaline propensities.

Arkövy's experiment was as follows: two teeth were accidentally left in a culture of B. gangrænæ pulpæ (B. mesentericus var. niger) and in some three months came to light by accident. They were both carious, and the reaction of the medium was alkaline. From this Arkövy deduced primary caries occurring in an alkaline medium.

In the superficial layers of carious dentine a large variety of species are constantly met with, some of them producing digestive enzymes, others producing acid fermentation, whilst some are capable of both functions. Among these liquefying bacteria some will dissolve fibrin and blood serum, others only gelatin. The ones dissolving blood serum I have found also capable of digesting decalcified dentine, while many of those only liquefying gelatin do not attack dentine (decalcified). Hard, undecalcified dentine I have never found attacked by the enzymes or bacteria. To determine the liquefying power of bacteria upon dentine thin strips of decalcified tooth are suspended in broth cultures of the organism to be tested. The strips of dentine should be well washed in dilute sodium carbonate and in distilled water after the lime salt has been removed by acids. To determine the presence of an enzyme a seven-days-old broth culture is poured into a tube containing decalcified dentine, and a crystal of thymol or a few drops of chloroform added to prevent further growth of the organisms; a control tube is also made containing sterile broth and thymol, with a strip of softened dentine. When an enzyme is present the dentine gradually dissolves but is unaffected in the control tube. To entirely eliminate the presence of organisms the culture may be filtered through a Pasteur-Chamberland filter and the filtrate containing the enzyme tested as before.

Many of these enzymes may be obtained fairly pure from a broth culture by precipitating them with two volumes of absolute alcohol filtering, and dissolving up the residue in thymol water. I have obtained on several occasions an active enzyme by this procedure capable of digesting dentine. The liquefaction of gelatin may be conveniently tested by making gelatin tubes containing 10 per cent. gelatin in saturated thymol water; the tubes do not require cotton wool plugs but are kept mouth downwards in water until wanted for use.

The culture to be tested is poured into one of these tubes,

and a crystal of thymol added to prevent further growth of the organisms; a control tube is also made containing thymol water alone. The tube containing the enzyme shows marked liquefaction in a few days.

I have not found liquefying organisms in the deep layers, with the exception of the staphylococcus albus, which organism is curiously influenced by its environment, so much so that the amount of liquefaction produced by one of the species isolated from the deep layers is so slow when first tested that it often requires a week or ten days to produce any definite liquefaction. Galloway and Eyre[1] have shown that by keeping a normal liquefying staphylococcus albus for long periods under strictly anäerobic conditions, the rate of liquefaction is reduced to less than a third of that of the control culture grown äerobically. The non-liquefaction of organisms isolated from the deep layers of carious dentine is no doubt explainable in this way.

So far I have not obtained anäerobic (obligatory) liquefying organisms from dental caries, all the liquefiers being facultative äerobic.

Choquet[2] has isolated five different organisms from the recurrent caries occurring underneath fillings of three teeth. Unfortunately these organisms are not described on the usual test media in use, and it is therefore impossible to determine if they are known species or not. Choquet does not appear to have found any of the organisms observed by other writers, but as he has adopted the use of special media *only*, such a fact is not altogether surprising.

Notwithstanding the profound modification that environment and food-stuffs may have upon the flora of the mouth, the organisms present in caries are fairly constant; and adopting the criteria of (*a*) acid production and (*b*) liquefaction as a rough division of the organisms concerned, we may arrange the bacteria of dental caries as follows:—

[1] Internat. Med. Congress, Paris, 1900.

[2] *Dental Cosmos*, October, 1900.

Bacteria of Dental Caries.

Acid-forming Bacteria.

Streptococcus brevis B. necrodentalis Staphylococcus albus	Deep layers of carious dentine.
Streptococcus brevis Sarcina lutea Sarcina aurantiaca Sarcina alba (Eisenberg) Staphylococcus albus Staphylococcus aureus	Superficial layers of carious entine.

Bacteria which liquefy Dentine (decalcified).

None isolated as yet ...	Deep layers of carious dentine.
B. mesentericus ruber B. mesentericus vulgatus B. mesentericus fuscus B. furvus B. gingivæ pyogenes B. liquefasciens fluorescens motilis B. subtilis Proteus Zenkeri B. plexiformis	Superficial layers of carious dentine.

Dobrzyniecki,[1] working in Arkövy's laboratory, gives the following list of organisms occurring in carious dentine which agrees closely with my own. B. gangrænæ pulpæ (Arkövy) is apparently a variety of B. mesentericus, probably "niger." Siberth and myself have both come to this conclusion independently, and we have both apparently failed to find the organisms described by Arkövy, but have constantly met with bacilli of the mesentericus group (potato bacilli).

Chief Bacteria of Dental Caries.

(Dobrzyniecki.)

B. gangrænæ pulpæ.
Staphylococcus aureus.
Streptococcus pyogenes (S. brevis ?).
Sarcinæ lutea.
Staphylococcus albus.

[1] *Cent. für Bakteriol.*, Bd. xxiii., 1899.

FIG. 48.—STREPTOCOCCUS BREVIS.
Agar cultivation at twenty-four hours. Stained Gram. × 1,000.

FIG. 49.—STREPTOCOCCUS BREVIS.
Twenty-four hours' broth cultivation. Stained Gram. × 1,000. (Washbourn and Goadby, *Odonto. Soc. Trans.*, 1896.)

I have only found the staphylococcus aureus occasionally, and generally the non-liquefying variety.

A considerable number of chromogenic bacteria are found associated from time to time in dental caries.

(17) STREPTOCOCCUS BREVIS (Van Lingelsheim).
Micrococcus nexifer (*Miller*).

Found in the mouth of normal persons as a constant inhabitant, generally around the epithelial cells as diplococci, rarely, if ever, in chains. Fig. 35.

Staining Reactions.—Stains by Gram's method and by the ordinary aniline dyes. The cocci are generally pear-shaped, and rarely show involution forms except in very old cultures.

Biological Characters.—An äerobic, facultative anäerobic, non-motile streptococcus. No pigment formed.

Gelatin Plates, 22° C.—In forty-eight hours minute grey-white flat colonies are formed, which increase slowly and never attain a large size. Microscopically: very faintly granular, deep colonies, lenticular or morula-like.

Gelatin Stab, 22° C.—Scanty flat growth upon surface and granular beaded growth along the needle track. Occasionally liquefaction said to occur.

Gelatin Shake, 22° C.—Three days: minute colonies scattered throughout medium.

Gelatin Streak, 22° C.—Fairly well-defined grey growth in forty-eight hours, with regular edge and minutely granular surface; colonies discrete. According to Lingelsheim, slight liquefaction of gelatin takes place, but I have not been able to confirm this.

Agar Streak, 37·5° C. — Well-marked growth in twenty-four hours of minute, moist, grey colonies with no marked centre and rapidly coalescing.

Potato, 37·5° C.—Forty-eight hours: a shining patch of growth is seen which may become well marked. No pigmentation occurs.

Litmus Milk, 37·5° C.—Twenty-four hours: solid clot with well marked acid reaction. The greater part of the clot is decolourised with the exception of the upper portions. The clot does not become re-dissolved.

Broth, 37·5° C.—Well marked general turbidity in twelve hours; no flocculi are found in the fluid, but a slight granular deposit is formed. No indol is produced. No H_2S.

Blood Serum, 37·5° C.—Similar to agar.

Broth containing glucose, maltose, lactose, dextrin, inulin, &c., shows a marked acid reaction in twenty-four hours. The acidity persists for an indefinite time.

Pathogenesis.—Not pathogenic for guinea-pigs and rabbits when injected subcutaneously or intraperitoneally.

(18) SARCINA LUTEA.

Found in similar situations to the last species. Very common in the air. Often present in the mouth and throat.

Morphology.—Cocci 1 to 1·5 μ in diameter associated in pairs of hemispherical form, or in packets of four or eight. Sarcina form, generally well marked.

Biological Characters.—An äerobic, liquefying, chromogenic sarcina.

Staining Reactions.—Stains by the ordinary aniline dyes and by Gram's method. No endogenous spores are formed but arthrospores have been described (Hueppe).

Gelatin Plates, 22° C.—In two to three days spherical yellow colonies appear with projecting central portion, moist, entire and opaque. Under the $\frac{2}{3}$ obj. the colonies are coarsely granular and with irregular edge. The colonies grow slowly and do not commence to liquefy for four or five days.

Gelatin Stab, 22° C.—A coarsely granular growth of isolated colonies (filiform and beaded) along the stab, the colour turning a deep yellow. The surface generally shows a distinct button of growth before liquefaction commences. The liquefaction occurs first as a funnel-shaped depression and gradually spreads to the tube walls, flocculi of a yellow colour fall to the bottom of the funnel and form a granular deposit.

Some varieties do not liquefy (Chester).

Gelatin Streak, 22° C.—A well marked layer is formed which does not liquefy the medium for several days.

Agar Streak.—A well marked canary-yellow moist opaque growth is formed in three days at 22° C. At 37·5° C. the colour is not so pronounced, and the growth tends to a dirty yellow-white, and is moist and slightly viscous.

Broth, 37·5° C.—Little turbidity with yellow deposit in twenty-four hours. Indol slight; a little H_2S.

Litmus Milk.—Coagulation of the casein occurs in two days, the clot being slowly re-dissolved; the reaction is strongly acid.

Potato.—Raised, glistening and limited to streak, surface rough and sulphur-yellow in colour.

Glucose Broth.—Slight acid production.

(19) SARCINA AURANTIACA.

Found in air and common in carious teeth, and in gingival inflammations.

Morphology.—Cocci 1 to 1·5 μ in diameter, varies considerably, occurring typically in packets of eight, but also occurs in pairs of large hemispherical elements and in groups of four (tetrads).

The elements are smaller than those of S. lutea.

Staining Reactions.—Stains with the ordinary aniline dyes and by Gram's method. Sometimes evidences of a capsule may be observed.

Biological Characters.—An äerobic facultative anäerobic sarcina, growing in the usual culture media at the room temperature and forming an orange pigment. Arthrospores said to be formed.

Gelatin Plates, 22° C.—In forty-eight hours minute spherical granular colonies make their appearance. Under the $\frac{2}{3}$ obj. the deep colonies are seen to be somewhat of morula form and may become surrounded with a bubble of liquefied gelatin. The surface colonies are often two millimetres in diameter, moist, orange yellow in colour, raised and often with a central projection (umbonate).

Gelatin Stain, 22° C.—In two to three days a beaded orange growth occurs along the line of puncture, the separate colonies ultimately become confluent. Liquefaction commences at the surface and takes the form of a deep funnel; the apex is occupied with a granular orange-brown deposit and a good deal of flocculent material appears in the fluid. Eventually a scum forms on the surface of the liquefied gelatin.

Gelatin Streak, 22° C.—Liquefaction occurs in three or four days; before this occurs the growth is orange in colour and granular.

Agar, 37·5° C.—A golden yellow, moist, shining layer is formed, the colour being darker than that produced by the staphylococcus aureus, and the growth more pronounced and moist.

Potato, 22° C.—Development occurs slowly and is chiefly confined to the line of inoculation, and is of brown-yellow colour.

Blood Serum, 37·5° C.—A well marked layer of dark brown colour is formed; the blood serum may be liquefied in old cultures.

Broth, 37·5° C.—In twenty-four hours a well marked general

turbidity, large flocculi and a thick granular deposit of a brownish-orange colour. Indol slight; no H_2S.

Litmus Milk.—In forty-eight hours a well marked acid reaction is present and the casein becomes coagulated and later re-dissolved.

Glucose Broth.—Acid production slight.

(20) BACILLUS MESENTERICUS VULGATUS.

Found widely distributed in milk, water, alimentary tract (Vignal), carious dentine, gangrenous pulps, dento-alveolar abscesses and gingival inflammation.

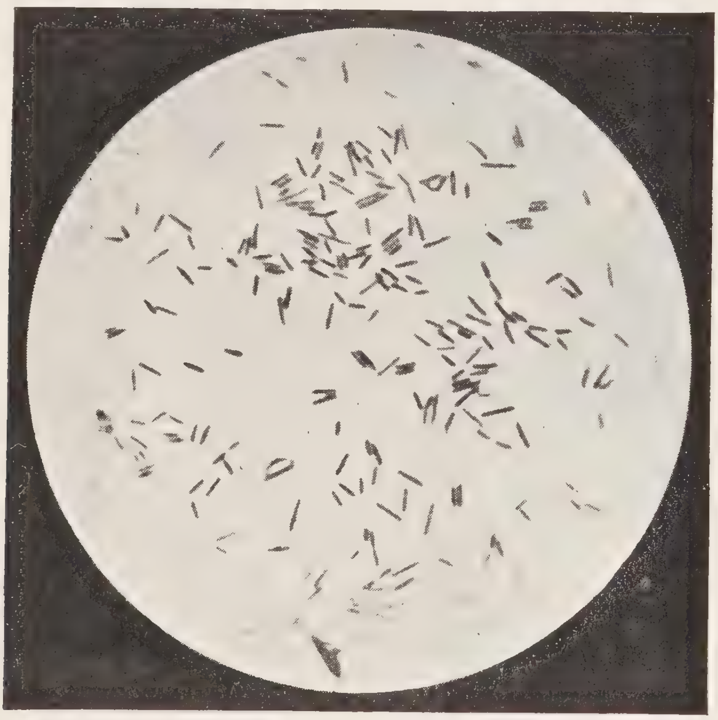

FIG. 50.—BACILLUS MESENTERICUS VULGATUS.
Twenty-four-hours-old cultivation in agar. × 1,000.

Morphology.—Bacilli 1·2 to 3 μ long, 0·75 μ wide, ends rounded, generally associated in pairs and in chains (streptobacilli). Often long threads are formed, well marked oval spores found, generally at ends of bacilli.

Staining Reactions.—Stains with carbol-methylene blue and usual stains, and by Gram's method. Spores stain with hot carbol-fuchsin in the usual way.

Biological Characters.—An äerobic, facultative anäerobic, liquefying, motile bacillus, forming spores.

Gelatin Plates, 22° C.—In forty-eight hours the colonies are transparent, bluish white, but later become opaque with a white centre. Liquefaction progresses rapidly, and in three days the superficial colonies, which may have attained a diameter of 1 centimetre, are floating in the liquefied gelatin. Under the $\frac{2}{3}$ obj. they appear granular and with irregular margins, later the surface becomes wrinkled and irregular.

Gelatin Stab, 22° C.—In forty-eight hours a small funnel-shaped area of liquefaction has formed. Liquefaction progresses rapidly, and in three days has extended to the tube walls. The funnel-shaped depression is still seen in the lower part. Flocculi appear in the fluid, and a wrinkled pellicle is formed on the surface. The funnel generally remains clear except at the apex, which is filled with granular deposit.

Gelatin Streak, 22° C.—Liquefaction commences in twenty-four hours and rapidly progresses, delicate flocculi being formed in the fluid.

Agar Streak, 37·5° C.—A well marked moist dirty grey streak with irregular edges is formed in twenty-four hours; in forty-eight hours the surface becomes covered with a wrinkled layer. The medium is not stained.

Blood Serum, 37·5° C.—In twenty-four hours a well marked groove of liquefaction is formed, which increases rapidly. The growth is moist and viscid. Some darkening of the medium may occur.

Potato, 22° C.—A thick wrinkled white layer is formed all over the surface, and generally extending to the back of the slice. The culture is thick and stringy, and may be drawn out into threads with the platinum needle.

Broth, 37·5° C.—In twenty-four hours a slight precipitate is formed, the fluid remaining clear; later a thin wrinkled pellicle is formed on the surface of the medium, and the deposit becomes slightly viscid. The medium does not become turbid. The organism will grow in 1 in 200 of hydrochloric acid (Vignal).

Litmus Milk, 37·5° C.—In twenty-four hours no change; in forty-eight hours coagulation of the casein takes place, but no acid is formed. The casein becomes re-dissolved and floats up to the surface as a slimy layer, the fluid becoming clear, whilst a good deal of unpleasant smell is produced. Reaction alkaline.

(21) BACILLUS MESENTERICUS RUBER.

Found in company with the foregoing.

Morphology.—Bacilli 1·2 to 3 μ long, 0·5 μ wide, slightly more slender than B. mesentericus vulgatus, but like that organism forms streptobacilli and threads. Forms oval spores which are highly resistant to heat and germicides.

Staining Reactions.—Stains with the usual aniline dyes, and by Gram's method.

Biological Characters.—An äerobic, liquefying, motile, chromogenic bacillus. Forms spores.

Gelatin Plates, 22° C.—At the end of forty-eight hours the superficial and deep colonies are different. The former have a spreading edge with fine ramifications passing out into the medium, the deep colonies are yellow in colour and spherical in shape. When they reach the surface they spread out in a similar fashion to the deeper ones. Liquefaction commences about the third or fourth day, and the colonies float on the surface of the fluid, the radiating process disappearing, and the surface becoming dark and wrinkled. The medium becomes stained a light brown.

Gelatin Stab, 22° C.—In three or four days growth has developed to the bottom of the stab, liquefaction commencing as a funnel-shaped depression at the surface, and rapidly spreading to the walls of the tube (saccate). The funnel remains below the liquefied gelatin, flocculi forming as in the other members of the group.

Gelatin Streak, 22° C.—In two days a well marked liquefied groove is formed.

Agar Streak, 37·5° C.—A dirty yellow or white crumpled layer is formed all over the surface in twenty-four hours, which becomes dry in old cultures.

Blood Serum, 37·5° C.—In two days well marked liquefaction has taken place, the medium becoming darkened.

Potato, 37·5° C.—In twenty-four hours a viscid yellowish-white or pink layer has formed covering the whole surface of the slice; in two days the growth has extended round the whole of the potato, which is coloured a rose pink. The extension to the back of the slice is characteristic. At 22° C. the layer is more yellow, viscid, and does not turn pink.

Broth, 37·5° C.—Flocculent deposit in twenty-four hours, later a slight pellicle is formed which has a brown wrinkled surface.

Litmus Milk, 37° C.—Coagulation of the casein occurs in two

to three days, the coagulum is re-dissolved, and the fluid clears. Grows at any temperature from 15° to 40° C.

(22) BACILLUS MESENTERICUS FUSCUS.

Found in similar situations to the last.

Morphology.—Bacilli 1·3 to 3·5 μ long, 0·5 μ broad, ends rounded; forms chains and threads.

Staining Reactions.—Stains with the ordinary aniline dyes, and by Gram's method. Spores stain in the usual way.

Biological Characters.—An äerobic, liquefying, motile, spore-forming bacillus. The spores are irregularly placed in the rods, and are rather smaller than those of the other two species.

Gelatin Plates, 22° C.—In two days spherical white colonies are found which have radiating processes extending into the medium. The superficial colonies particularly have a fine granular surface. Liquefaction rapidly occurs, the medium turning brownish in tint.

Gelatin Stab, 22° C.—In forty-eight hours a beaded growth appears to the bottom of the stab. Liquefaction commences at the surface in a funnel shape and rapidly reaches the walls of the tube. Flocculi form in the medium, and a pellicle on the surface.

Gelatin Streak, 22° C.—A liquefied groove appears in two to three days filled with flocculi.

Blood Serum, 37·5° C.—A groove of liquefaction with discolouration of the medium occurs in two days.

Potato, 37° C.—A well-marked rugose yellowish-brown layer is formed of wash-leather colour; the potato becomes stained. The growth is thin and does not penetrate so much as does B. mesentericus ruber or vulgatus. A good deal of sour putrefactive odour is given off from the potato and blood serum cultures.

Broth.—37·5° C.—A flocculent precipitate is formed, and later a surface pellicle, brown and wrinkled.

Litmus Milk, 37·5° C. — Coagulation of casein occurs, the coagulum eventually becoming re-dissolved and floating on the surface of the fluid, which clears. The reaction is alkaline, the fluid turning a bluish-brown. Some gas is formed.

(23) BACILLUS LIQUEFASCIENS FLUORESCENS MOTILIS.

(*Bacterium termo of Vignal.*)

Found in water and in various putrefying infusions; often present in the mouth and sputum.

Morphology.—Bacilli 1·5 to 2 μ long, and 0·3 μ broad; often united in pairs with a central constriction; grows out into filaments.

Staining Reactions.—Stains with the ordinary aniline dyes, but not by Gram's method.

Biological Characters.—An äerobic, facultative anäerobic, chromogenic, liquefying, motile bacillus; does not form spores.

Gelatin Plates, 22° C.—In forty-eight hours white colonies are formed which may attain a diameter of 2 mm., a ring of liquefied gelatin forming around each; under $\frac{2}{3}$ obj. the colonies have a well-defined, sharp outline with a circular dentate edge. The centre of the colony is darker and brown in colour, finely granular; the outer zone is paler and yellow in colour, finely granular, and becoming translucent towards the edge. The surrounding gelatin gradually becomes a fluorescent green by transmitted, yellow by reflected light.

Gelatin Stab, 22° C.—A white granular growth quickly appears along the line of puncture, a small funnel of liquefied gelatin appearing at the surface, the widest part of which is usually occupied by an air bubble. The liquefaction gradually extends to the sides of the tube and at the same time in a downward direction, the liquefaction ultimately taking place in a horizontal plane (stratiform). Just below the area of liquefaction, and spreading into the surrounding medium, is a delicate fluorescent green colouration which gradually spreads throughout the tube. The colour is green by transmitted, yellowish-brown by reflected light. A thick white deposit is formed at the bottom of the liquefied medium.

Agar, 37·5° C.—A slimy, moist, grey-white layer is rapidly formed; at 22° C. the fluorescent green pigment is produced as on gelatin and colours the whole of the medium, which later becomes of a dirty yellow-brown. The growth itself is not coloured.

Potato, 22° C.—A well marked brownish layer is developed and the potato stained a dark tint. The fluorescent green colour may be extracted with chloroform from the potato cultures.

Blood Serum, 37·5° C.—Three days: dark and discoloured groove of liquefaction.

Broth.—37·5° C.—Twenty-four hours: general turbidity with thick precipitate. Indol is formed in small quantities, but no H_2S.

Litmus Milk, 22° C.—Not coagulated, but clears up gradually with yellowish-green colouration.

Bacillus fluorescens non liquefasciens is also often found in the mouth. As its name implies, it does not produce liquefaction of gelatin or blood serum, and is non-motile. The green fluorescent pigment produced is often seen in broth cultivations, especially so when the culture is shaken up with air, the colour disappearing again on standing.

The other biological reactions are similar to the organism described above. Ruzicka,[1] after a prolonged examination of this group of bacteria and the B. pyocyaneus, came to the conclusion that they were all varieties of one species, although perhaps modified by their surroundings, &c. Lehmann and Neumann[2] also support this view. There are a considerable number of organisms described as producing a greenish fluorescence, and it is certainly not improbable that they are also members of this one species.

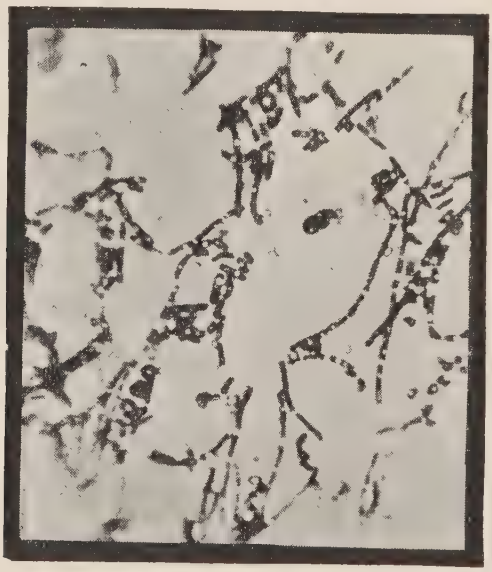

FIG. 51.—BACILLUS SUBTILIS, SHOWING SPORE FORMATION. × 1,000.

(24) BACILLUS SUBTILIS. (*The Hay Bacillus.*)

Found in soil and water, very common. Often associated with the Mesenteric group in the mouth and in carious dentine.

Morphology.—Bacilli 4·6 to 6 μ long, 0·75 to 1 μ broad, with rounded ends. Often associated in chains (streptobacilli). Sometimes grows out into very long filaments, especially in liquid media. Flagella multiple (peritrichic).

---

[1] *Arch. für Hygiene*, Bd. xxxiv., p. 148.
[2] Bacteriology, p. 286.

Staining Reactions.—Stains with the ordinary aniline dyes, and by Gram's method. The spores stain well by Möller's method. The flagella are stained by Pitfield's method.

Biological Characters.—An äerobic, liquefying, motile bacillus. Forms spores, which are generally situated at the ends of the rods. The spore germination is characteristic. The germinating spore splits along one side, and the organism grows out through the rent, the remains of the spore often remaining attached to the end of the bacillus. The motility is of a curious waddling nature, and is not very rapid even when the bacillus possesses many flagella.

Gelatin Plates, 22° C.—In twenty-four hours minute grey-white colonies appear under the $\frac{2}{3}$ obj., they are granular, greenish, and have a well defined but irregular outline. Development progresses rapidly, and in two days well marked liquefaction has taken place, forming saucer-like cavities with granular, translucent contents; the central part being opaque and white. Under the $\frac{2}{3}$ obj. the colonies are greyish-yellow in the centre, and greenish-grey towards the periphery where a tangled mass of filaments is to be seen, which radiate into the surrounding medium and also into the non-liquefied portion (crateriform, turbid, entire, becoming ciliate).

Gelatin Stab, 22° C.—A white growth rapidly appears along the needle track (saccate), and liquefaction soon commences with the formation of a wrinkled mycoderma upon the surface. The pellicle thus formed sinks to the bottom of the liquefied gelatin, and is replaced by another which in turn sinks, so that a thick deposit is formed at the bottom of the tube. The fluid which at first is filled with white flocculi becomes clear as the result. Occasionally the liquefaction does not progress so rapidly, and fine radiating processes extend into the non-liquefied medium, which disappear as liquefaction progresses. Various races of subtilis show considerable variation in their liquefying power. No gas is formed.

Agar Streak, 37·5° C.—In twenty-four hours a grey, opaque flat growth with defined edges, which later becomes dry, irregular and brownish in colour. The whole may be often lifted away from the surface of the nutrient medium. The surface, at first slightly mottled, becomes corrugated and wrinkled (crumpled).

Potato, 37·5° C.—In twenty-four hours the whole surface of the slice is covered with a moist creamy growth which extends on to the glass of the culture tube. The growth is found to be full of spores when examined microscopically.

Blood Serum, 37·5° C.—A wrinkled mycoderma is rapidly formed over the surface, and liquefaction of the medium occurs.

Broth, 37·5° C.—In liquid media general turbidity is rapidly formed, and the characteristic wrinkled mycoderma is formed upon the surface, and becomes firmly attached to the tube walls. On vegetable infusions of all kinds a similar wrinkled pellicle is rapidly formed, and is seen to consist of tangled threads when examined under the microscope. Indol negative.

Litmus Milk.—An acid reaction is generally produced, and the casein is dissolved. Later the reaction becomes alkaline. Various sugars are oxidised, saccharose inverted and then oxidised. The process is continuous if the acid formed is neutralized as formed with precipitated chalk (Lefar).

The organism is extremely sensitive to acids, and in the presence of minute traces forms all sorts of curious involution forms (see fig. 3).

(25) PROTEUS VULGARIS.

The common bacterium present in putrefactive processes; widely distributed. This and other putrefactive bacteria were once described under the term "Bacterium Termo."

Morphology.—Bacilli with rounded ends, 0·8 μ broad, of variable length (1·5 to 3 μ); long filaments 20 μ, and longer are formed; these filaments may be flexible and sometimes spiral in form. The rods are frequently united in pairs; they are motile and have peritrichic flagella (fig. 3 (iii.) *a*). Involution forms are common, the most frequent being of globular shape, and are generally found in cultures incubated at 37·5° C. Spore formation not observed.

Staining Reactions.—Stains well with the ordinary aniline dyes, but does not retain the stain of Gram's method.

Biological Characters.—An äerobic, facultative anäerobic, liquefying, motile bacillus growing in the usual culture media at the room temperature. Optimum temperature 22° C.

Gelatin Plates, 22° C.—In 5 per cent. gelatin in eight hours small depressions are seen on the surface of the medium. Under the microscope these depressions are seen to have amœboid-like processes extending on to the surrounding surface. These processes undergo constant change in form, and may become detached and wander over the plate (Sternberg).

The deep colonies have radiating processes extending into the

unliquefied gelatin. No wandering colonies are formed in 10 per cent. gelatin.

Gelatin Stab, 22° C.—Liquefaction takes place to the bottom of the puncture and rapidly spreads to the walls of the tube (saccate); near the surface a white cloudiness is produced and an abundant flocculent deposit is formed at the bottom.

Gelatin Shake, 22° C.—Abundant gas formation and liquefaction of gelatin.

Blood Serum, 37·5° C.—Dirty white growth in twenty-four hours with well-marked liquefaction of medium, which becomes discoloured.

Agar, 37·5° C.—In twenty-four hours a spreading, pale yellowish, glistening, translucent growth covering the whole surface of the medium.

Potato, 37·5° C.—In twenty-four hours a dirty white moist growth. The surface of the potato becomes dissolved. Putrefactive smell and alkaline reaction.

Litmus Milk, 37·5° C.—Slightly acid in twenty-four hours, later distinctly alkaline, but no clotting takes place. The casein becomes dissolved, and the fluid ultimately is clear with a thick precipitate of a brown-blue or yellow colour.

Broth, 37·5° C.—General turbidity and precipitate in twenty-four hours; there is generally a putrefactive smell. Indol formed. H_2S present.

Nitrate Broth.—Well marked reaction with naphthylamine (see page 226).

Glucose Broth.—Gas formed $\frac{H}{CO_2} = \frac{2}{1}$; no gas in lactose broth.

Pathogenesis.—Pathogenic for rabbits and guinea-pigs when injected into the veins in considerable quantities. Cheyne estimated that a cubic cm. of liquefied gelatin contained 4,500,000,000 bacilli, and that a smaller dose than 9,000,000 produced no ill effect. The organism has often been isolated *post mortem* from venous thrombi.[1] Filtered cultures cause toxæmia.

(26) BACILLUS NECRO-DENTALIS (GOADBY).

Found in dental caries, especially in the deep layers of carious dentine, where the rods may be found blocking up the dentinal canal. When first isolated it grows best in an atmosphere free from

[1] Bryant, *Trans. Path. Soc.*, 1901.

oxygen, but is facultative äerobic, and grows well under ordinary conditions. It develops well on the ordinary laboratory media used.

Morphology.—Bacilli 0·75 μ broad, and 1 to 5 μ long, often associated in pairs and sometimes in chains (streptobacilli). The ends of the bacilli are square or rounded. In anäerobic cultures the bacilli tend to grow out into long threads; under äerobic conditions the organism is much shorter. The bacilli tend to involute rapidly, and form swollen and contorted masses not unlike the streptococcus.

FIG. 52.—BACILLUS NECRODENTALIS.
Agar cultivation, forty-eight hours. Stained Gram. x 1,000.

In broth cultures the bacilli are very short and appear more like cocci. They are slightly motile, best marked on anäerobic cultures. I have not succeeded in staining the flagella.

Staining Reactions.—Stains by the ordinary aniline dyes but takes some time, especially with methylene blue. It retains the stain of Gram's method. The involution forms stain badly and appear granular, but no polar staining has been observed.

Biological Characters.—An anäerobic, facultative äerobic, non-liquefying motile bacillus. No spore formation observed. Non-chromogenic.

Gelatin Plates, 22° C.—In three days minute colonies appear, not much larger than a pin's point. The colonies spread a little, and feathery processes extend into the medium. The deep colonies are often surrounded with a series of fine rays. No liquefaction of the gelatin occurs.

Gelatin Streak, 22° C.—In three days a slight beaded growth occurs, which later sends processes into the medium. The gelatin is not liquefied, but may become a little softened around the colonies.

Gelatin Stab, 22° C.—Slight granular growth along the line of puncture, radiating processes may be formed. No liquefaction occurs.

Gelatin Shake, 22° C.—No gas bubbles are formed.

Potato, 37·5° C.—In forty-eight hours slight shining appearance upon the inoculated surface. The organisms show considerable involution.

Agar Plates, 37·5° C.—Minute grey colonies in forty-eight hours, round and regular or erose edge, and central nucleus brownish and raised. Microscopically, brownish-yellow with central nucleus faintly granular and regular edge. Under anäerobic conditions the colonies are larger.

Broth, 37·5° C.—In twenty-four hours slight general turbidity with a flocculent precipitate, which increases whilst the turbidity does not. No pellicle is formed. No indol produced in ten days; no H_2S formed.

Litmus Milk, 37·5° C.—In twenty-four hours no change; in forty-eight hours solid clot, lower portion decolourised, the top showing a marked acid reaction. The clot is not re-dissolved.

Glucose broth, lactose broth, starch broth, maltose broth: strong acid reaction in forty-eight hours.

Anäerobiosis.—Well marked growth on glucose formate agar in Buchner tubes. The colonies are much larger than on äerobic media, and are brownish in colour and have a well marked nipple-like central projection (umbonate). No gas is produced on glucose formate broth, but the turbidity is well marked.

Pathogenesis.—Not determined.

Spore Formation.—No spores stainable. Cultures three weeks old, heated to 70° C. for half an hour, gave no subsequent growth.

Optimum temperature, 37·5° C. Thermal death point 60° C.

FIG. 53.—BACILLUS PLEXIFORMIS (GOADBY).
Gelatin cultivation at forty-eight hours. x 1,000.

FIG. 54.—BACILLUS PLEXIFORMIS.
Twenty-four hours' cultivation of decalcified dentine. x 1,000.

(27) BACILLUS PLEXIFORMIS (Goadby).

Found occasionally in carious dentine.

Morphology.—Curved and twisted bacilli on most media ; may be associated in pairs or grow out into long threads 30 μ or more long. In gelatine cultures the bacilli are short and tend to stain irregularly (see fig. 53), while on slices of decalcified dentine long threads are formed (see fig. 54). Motility well marked but flagella not stained.

Staining Reactions.—Does not stain by Gram's method, stains by ordinary aniline dyes. No spores observed.

Biological Characters. Gelatin Plates, 22° C.—Minute white to grey colonies (punctate or effused). Gradual liquefaction occurs.

Gelatin Stab, 22° C.—Filiform growth to bottom of stab, liquefaction only in upper part. Stratiform, well marked flocculent deposit and general turbidity of fluid.

Gelatin Streak, 22° C.—Well marked liquefied groove in two to three days.

Agar Plates, 37·5° C., 2 to 3 mm.—Translucent colonies raised and round (pulvinate), edge entire, of rather viscous consistency.

Agar Streak, 37·5° C.—Raised, moist, gummy, confined to needle track.

Blood Serum, 37·5° C.—Dirty brown streak, eventually slight liquefaction.

Potato, 22° C.—Brownish slimy growth, slow in appearance, confined to streak. Not spreading.

Litmus Milk, 37·5° C.—Slightly alkaline reaction, no clot.

Broth, 37·5 C°.— General turbidity with somewhat flocculent deposit, no pellicle. Indol reaction well marked.

Glucose formate media.—No anäerobic growth and no gas bubbles formed.

CHAPTER IX.

Bacteria in Tooth Pulps.

The channel of infection of the tooth pulp is along the dentinal canals, and may occur with but slight and almost imperceptible signs of caries in the tooth surface (see fig. 47, inroads of organisms well shown).

When once the dentine has been reached the organisms are able to make their way along the dentinal tubules, at the same time that their products penetrate to the pulp surface by capillarity. I have often found that cultivations and microscopical examination of pulps which had succumbed to caries showed no bacteria, while the dentine at a short distance from the pulp chamber gave positive results. Miller has several times pointed out that cultivations made from tooth pulps gave no evidence of living organisms even after most careful examination. The soluble products of bacterial activity may therefore produce death of the pulp, accompanied with fatty or other degeneration, without the living organisms themselves actually coming into play, and the familiar clinical observation of the ease with which arsenious acid gains access to the pulp demonstrates the permeability of sound dentine.

In the largest number of cases, however, bacteria are present in dead pulps; some of them have been found by Miller to be pathogenic for animals, generally producing local necrosis or suppuration when injected subcutaneously. Miller examined fifty cases of pulp gangrene, and notes several of the organisms met with. Many of these are gas-forming bacteria, and most of them capable of fermenting carbohydrates. Among the known pathogenic bacteria that have been found in tooth pulps, the streptococcus has been observed by most of the workers: Miller,[1] Siebeth,[2] Dobrzyniecki[3] and myself have constantly met with it, but I am inclined to think that it is the

[1] *Dental Cosmos*, July, 1894.

[2] *Central. für Bak.*, 1900, xxviii., p. 302.

[3] *Central. für Bak.*, 1898, xxiii., p. 670.

ordinary streptococcus of the mouth (S. brevis) rather than the pathogenic streptococcus.

Miller also met with Micrococcus tetragenous, whilst Zierler[1] occasionally found sarcinæ.

All these organisms are met with from time to time in carious dentine, so that their presence in dead pulps is not surprising, and I can confirm the occasional presence of both sarcinæ and micrococcus tetragenous from my observations. Staphylococci are not infrequent, the most common variety being the Staphylococcus albus, although the staphylococcus aureus does also occur. I have occasionally (four times), met with S. aureus in pure cultivations in the abscesses of roots in which the pulp had been dead for a considerable time. B. necrodentalis is also often obtainable, as is B. gingivæ pyogenes. The cultural characters of these bacteria have been already given (pages 127 and 161) in the chapter on Dental Caries.

Miller, in the paper already referred to, gives the following list of four organisms which he has frequently met with :—

(1) Cocci and diplococci (pathogenic).

(2) Bacilli, curved and often forming threads.

(3) Short rods with bipolar staining.

(4) Micrococcus tenuis.

He found these organisms frequently present, but does not state how often in pure culture, or in combination with the others.

A number of other organisms were also observed, some of which grew upon agar, others upon gelatin, but it is not clear which. The cultural reactions were unfortunately omitted.

Miller concluded that the cocci present were probably more concerned in pulp destruction than the bacilli, but that some symbiotic relation existed between the bacilli and cocci; injection of animals with mixed cultures producing more marked effect than the pure cultures alone. Inoculation with masses of putrid pulps generally resulted in the death of the animals (mice and rats) in three or four days, with local tissue necrosis and occasionally septicæmia.

Miller did not meet with the pneumococcus during any of his investigations; although more than one hundred and fifty mice were inoculated with putrid pulps, in no case did the pneumococci appear in the animals' blood after death.

Schreier[2] claims to have observed the pneumococcus in dead

[1] *Med. Rundschaw Ber.*, 1900, p. 534.

[2] *Oest. Vng. Vietelj. für Zahnarzte*, 1893, Heft ii.

pulps; two experiments only were made, and the inoculations performed with two broth cultures; one animal died, the others did not.

From the experiments of Miller it is extremely improbable that the pneumococcus is a constant organism in the diseased dental pulp, and from the particulars given by Schreier it is also highly improbable that the organisms he isolated were pneumococci.

Arkövy, Zierler and Sieberth have all noted the presence of bacilli of the Mesentericus group (potato-bacilli) in the gangrenous pulps. Arkövy first described an organism which he named B. gangrænæ pulpæ as constantly present in putrid pulps, the description appearing in the *Cent. für Bak.*, Bd. xxiii., p. 962, 1897, and he there described the organism in question as belonging to the Proteus group on account of its pleomorphism, in that it formed cocci on one medium and bacilli on another. Unfortunately further research resulted in the discovery that the supposed cocci were spores.

The supposed cocci were however originally figured stained by methylene blue, although they were said to stain but lightly. As the method of spore staining and resistance to high temperatures was also given, it is difficult to understand how the mistake arose, particularly as spores can be easily observed in hanging-drop specimens with a $\frac{1}{6}$ obj. on account of their highly refractile nature. Arkövy[1] in a later paper alters his classification of the organism and admits it belongs to the Subtilis group.

Zierler, a little later than Arkövy, described an organisism which Arkövy is at some pains to point out differs from B. gangrænæ pulpæ in that it does not produce so much colour, and that the liquefaction of gelatin is slower, while upon potato the organism described by Zierler produces a rose tint, whereas B. gangrænæ pulpæ turns the medium dark brown.

Otto Sieberth and myself have both noted the constant presence of bacilli of the Mesentericus group in pulps and carious dentine, and Sieberth is strongly of the opinion that B. gangrænæ pulpæ is one of the same group, and I quite agree, for in the bacteriological examination of a large number of dead pulps I have not met with any bacilli with such frequency as the Mesentericus group. Three marked varieties of this group are to be met with: (1) B. mesentericus vulgatus; (2) B. mesentericus ruber;

[1] *Cent. für Bakteriol.*, Bd. xxix., No. 19, p. 745.

(3) B. mesentericus fuscus. The last corresponds in all particulars to B. gangrænæ pulpæ, whilst the second is without doubt the organism found by Zierler.

The spores of these organisms are highly resistant and withstand boiling for half an hour or more.

The biological description of the "Potato bacilli," as they are termed, is given as well as that published by Arkövy of B. gangrænæ pulpæ.

CHAPTER X.

Bacteria in Dento-Alveolar Abscesses.

The bacteria found in the acute abscesses arising from dead pulps belong as a rule to the cocci, but it is somewhat remarkable that the majority of cases do not give cultivations of the common pus cocci.

Staphylococcus aureus is the least often met with in my experience. In twenty cases examined carefully S. aureus occurred in three, and on another occasion it was present in pure cultivation in the pus of a dead tooth, only a slight local inflammation being present, with little or no swelling and no pointing through the alveolus.

There appears to be more than one organism concerned in the process that may, after gaining access to the alveolus by way of the pulp chamber, produce a chronic inflammatory condition of the tissues in the region of the apex, remaining more or less quiescent until some secondary cause stimulates them into activity. One organism in particular, a coccus, I have frequently found in pure culture in abscesses involving teeth. The determination of the organisms present in such abscesses as have not pointed through the alveolus is simple; the root canal and pulp chamber are sterilized with hot instruments and a wisp of sterile wool introduced, and a cultivation made from the apical portion in one or two days' time. The colonies of the coccus are extremely gelatinous upon agar, and are most difficult to remove except *en masse.* The organism is described below.

Staphylococcus albus is the next most frequent organism met with in dento-alveolar abscess, and occurs in about half the cases, whilst Micrococcus tetragenous may also be sometimes found.

In some abscesses, more particularly those severe forms resulting in pyæmia or cellulitis, streptococci have been found by several

observers;[1] whilst in a series of twenty cases I examined consecutively, B. coli was present in two with particularly fœtid pus. A variety of alveolar abscess occurs occasionally around those teeth affected with pyorrhœa alveolaris; in these cases the coccus I have described below is frequently found, as are many of the other bacteria present in the exudate occurring along the gum margins. Kirk[2] made an investigation of the bacteria present in the small abscesses occasionally associated with living teeth. He found in some cases a diplococcus which had many of the characters of the pneumococcus, from which organism however it differed in many respects. The matter requires further investigation.

A variety of dento-alveolar abscess, fortunately not common, has a great tendency to spread and infiltrate the surrounding tissues. I have investigated four abscesses of this sort, but have been unsuccessful in isolating an organism which appears to be constantly present. It is anäerobic, and on the glucose formate stabs produces a considerable quantity of gas, so much so that the agar is often broken up and blown to the top of the tube. The microscopical examination shows bacilli forming long threads and showing a tendency to stain irregularly with methylene blue. As the organism was not isolated it is impossible to make any further statements. Staphylococcus albus was the only organism obtained in pure culture from these four cases. One other series of micro-organisms often associated with dental abscesses deserves mention, namely, the *Blastomycetes*.

It is not at all uncommon to find yeasts present in the chronic abscesses associated with dead roots which have been for a long time exposed to infection from the mouth, such roots, for instance, as have widely open root canals. So far I have not investigated the species, but it is interesting to note that recently a good many observers have described pathogenic blastomycetes which are capable of curious tumour formation (*cf.* the one described by Klein in the *Trans. Pathological Society*, 1901). In chronic abscesses Arkövy has constantly found the organism B. gangrænæ pulpæ (? Mesentericus) already discussed.

Altogether the bacteriology of dento-alveolar abscess is by no means fully worked out, and the literature of the subject is extremely

[1] Roughton, *Trans. Odont. Soc.*, November, 1891.
[2] *Cosmos*, 1901.

small. In general it would appear that the bacteria associated in the process are as a rule cocci, but that any organism or organisms able to set up suppurative pulpitis might be a cause of the abscess developing later. It also seems highly probable that some organisms hitherto undescribed are concerned in some of the more virulent forms of alveolar suppuration.

That many organisms of a pathogenic nature gain access to the body through the portal of diseased teeth is admitted by most, and that such bacteria produce chronic inflammatory conditions of the cervical glands is also well known, but concise bacteriological knowledge of the process has yet to be gained.

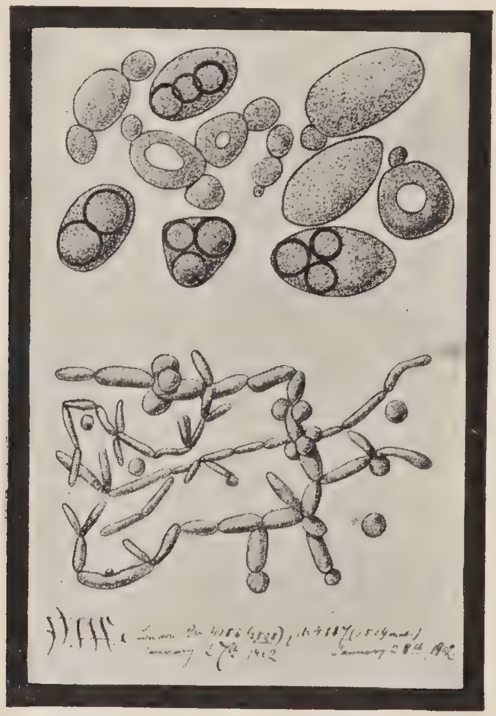

FIG. 55.—YEAST FORMS.
Showing development of mycelium (from Eyre's "Bacteriological Technique").

(28) STAPHYLOCOCCUS VISCOSUS (GOADBY).

Common in dento-alveolar abscesses and in suppuration along the gums; occurs occasionally in antral suppuration. Often associated with bacilli of Mesenteric group.

Morphology.—Irregular cocci, 0·75 to 1 μ wide. Sometimes arranged in fours, generally as isolated cocci or as staphylococci.

Staining Reactions.—Stains by ordinary aniline dyes and by Gram's method. There is a considerable amount of gelatinous

material around the organisms, which tends to stain with the ordinary dyes and is of a mucinous nature.

Biological Characters.—An äerobic, non-liquefying coccus; non-mobile. No spores formed.

Gelatin.—Does not grow at room temperature; grows slowly at 37° C.

Agar Plates, 37·5° C.—Flat spreading colonies, dentate edge, surface granular; microscopically, surface colonies, irregular dentate edge, brown to brownish-black, surface granular, marmorated (veined). Deep colonies, irregular, moruloid, brown.

Agar Streak, 37·5° C.—Two days: raised viscous shining streak, edge defined, wavy. The whole mass may be wound up upon the platinum needle and is extremely viscous.

Potato, 37·5° C.—Slight flat grey viscous growth confined to area of inoculation.

Blood Serum, 37·5° C.—Similar to agar; no liquefaction.

Litmus Milk, 37·5° C.—No change.

Broth, 37·5° C.—General turbidity with well marked pellicle and flocculi floating in fluid and deposit.

Peptone Water, 37·5° C.—H_2S formed. Glucose, dextrin, starch, lactose, acid in three days. No nitrite and no indol; gas evolved with nitrate media.

Glucose formate media, 37.5° C.—No growth anäerobically, but development when air is admitted

DISEASES OF THE MOUTH WITH UNDETERMINED BACTERIOLOGY.

Ulcerative Stomatitis.—Nothing is at present known of this condition as far as its bacteriology is concerned. It occurs with many diseases associated with fever and it appears to spread from the gum margins; particularly is this the case in the mouths of those already suffering from marginal inflammation and pyorrhœa alveolaris. Many of the mouth organisms are increased in numbers in the condition, especially the spirilla, and I can confirm Bernheim's[1] observations that these organisms are constantly present in the disease. Ulcerative stomatitis also occurs in an epidemic form,

---

[1] *Semaine Médicale,* 1897, p. 252.

and it has been suggested[1] that it is related to the "foot and mouth disease" of cattle.

Foot and mouth disease has been investigated by Löffler[2] and Trosch, who found that the cause was present in the vesicles and the mouth secretions. Moreover it required several filtrations through porcelain filters to remove the active agent, which so far is invisible to the most powerful microscopes. The lymph filtered once is still capable of producing the disease when inoculated into animals.

Aphthous Stomatitis is also without bacteriological investigation.

Gangrenous Stomatitis.—Petruschy[3] has found diphtheria bacilli together with pseudodiphtheria bacilli in two cases, which were cured with injection of diphtheria antitoxine. The condition occurs as a sequela of various fevers.

Mycosis of Tonsil and Mouth.—Occasionally large patches of a white numular nature are formed upon the tonsil and buccal mucous membrane. Sometimes these nodular masses are composed almost entirely of sarcinæ, at other times they are found to consist of tangled masses of threads (Leptothrix ?) and various other bacteria ; yeasts are also frequently present, sometimes to the extent of a false membrane. I have met with two such cases.

Epidemic Parotitis.—Laveran[4] and Mercay[5] and Walsh have found diplococci resembling staphylococcus albus in cases of mumps ; the injection produced transitory orchitis in rabbits and dogs, with occasional parotitis. The matter requires confirmation.

Suppurative Parotitis occurs occasionally associated with intestinal growth and gastric ulcer. In one case which came under my own observation staphylococcus aureus was present in pure culture, in another staphylococcus albus and a bacillus forming long threads which died out before its biology could be determined.

---

[1] Osler, "Princ. of Medicine," p. 442.
[2] *Cent. für Bakt.*, Bd. xxiii., 371.
[3] *Deut. med. Wochenschr.*, 1898, 600.
[4] *Comp. rend. Soc. Biol.*, 1893. [5] *Cent. für Bakt.*, xxi., 68.

CHAPTER XI.

Pyorrhœa Alveolaris.

Chronic suppurative periodontitis, caries alveolaris specifica, Rigg's disease, periostitis alveolo-dentalis, &c., &c., are among the terms applied by various authors to the chronic inflammatory condition of the gum margins and peridontal membrane leading to wasting of the alveolus and loss of the teeth. A coverslip preparation made from the pus found in the pockets around the teeth of chronic suppurative periodontitis shows a large number and variety of morphological forms so varied and changeable that there is considerable difficulty in tabulating them. Cocci as a rule are present in only small numbers in coverslip specimens, but in the usual culture media cocci invariably develop, even when plate cultivations are made from the mouth direct the majority of the colonies appearing belong to the genus cocci. Anyone who has been engaged for any length of time on the study of mouth bacteria cannot fail to have been struck with the difficulty of recovering in pure culture the organisms seen to be present in the pus of pyorrhœa. The *staphylococcus viscosus* described in the chapter on caries is frequently present, and from its constant presence in dento-alveolar abscesses may have some share in the pus formation. Most of the morphological forms met with develop for a period on maltose-agar, but it is well nigh hopeless to obtain pure cultures by the ordinary process of plating.

The organisms seen on direct examination may be tabulated as follows:—

(1) Cocci—generally in diplococci and massed around the epithelial cells in clumps.

(2) Thick bacilli generally jointed and often showing considerable irregularity in their staining characters.

(3) Thick bacilli with pointed ends and somewhat of the shape of a bean pod; they frequently show a division in the centre and appear as two elongated triangles with the bases opposed.

(4) Various fine bacilli 0·5 μ and under in width often exhibiting an irregular banded marking, especially well marked in the larger threads, which may be of great length.

(5) Spirilla, spirochæte, and comma-shaped bacilli, all showing marked motility in the hanging drop.

(6) Various yeast forms, sometimes with a partially developed mycelium.

(7) Streptothrix threads, generally showing well marked clubs (see fig. 67).

(8) Masses of bacilli associated with the threads, some jointed in chains, others free and often massed in clumps. Some of them exhibit polar-staining.

From pus containing all the above forms only the cocci develop with any degree of certainty when cultivated on artificial media.

In broth cultures the threads (4) may be obtained in impure culture, but I have only once succeeded in obtaining a pure culture, and even then the organisms died out before the proper cultural reactions could be ascertained.

It is obviously impossible therefore to say at present exactly which of the above organisms is especially related to the disease, or if the various morphological forms cited are only the various phases of one or two schizomycetes, or if the various forms are related to some higher class of organism. Until careful cultural experiments have resulted in a proper determination of these organisms the matter cannot be definitely decided.

Various observers have from time to time investigated the bacteria associated with pyorrhœa alveolaris, and of these Galippe and Miller deserve notice.

Galippe (1889) isolated two organisms which produced general abscess formation when injected into animals. With one the abscesses were frequently met with in the bones and were occasionally associated with spontaneous fracture, the site of the fracture being surrounded with a well defined area of suppuration. The second organism produced intermuscular abscesses. These organisms were however not properly described, and it is impossible to determine anything concerning them.

Miller conducted a series of cultural and inoculation experiments on the subject and came to only negative results. He cultivated a number of bacteria upon gelatin and agar from a large series of pyorrhœa cases, but was unable to satisfy himself that any par-

ticular organism isolated was the one chiefly concerned in the process. He made however several valuable observations, particularly the infrequency with which the common pus cocci were present. Thus in forty-three cases of pyorrhœa examined staphylococcus aureus was met with three times and staphylococcus albus twice.

Netter likewise found the pus cocci present in about 10 per cent. of the cases examined, and my own cultivation experiments confirm those of Miller and Netter, as in one hundred and fifty cases of marginal suppuration examined exactly 10 per cent. (15 cases in all) gave cultures of the staphylococcus aureus and albus.

So far my own experiments are very much in a line with those of Miller; I have isolated a large number of different bacteria, some of them pathogenic for animals, just as were a number of those obtained by Miller, but so far no organism appears with sufficient frequency to associate it especially with the disease. The results of some inoculation experiments, however, throw some additional light upon the subject. Animals (guinea-pigs) succumbed when inoculated with the filtrate of old broth cultivations, made from the mouth direct, and containing the fine threads referred to above, and moreover giving off a considerable fæcal smell. No organisms were found in the tissues at the *post mortem*, and it seems reasonable to suppose therefore that the animal died from a toxæmia, especially as there were evidences in the hæmorrhagic condition of the supra-renal capsules that such was the case.

Such a circumstance appears to point to a toxic element in pyorrhœa, and we may call to mind the curious shining atrophic appearance of the gums in cases of long standing. What appears therefore to be a reasonable supposition is that the particular bacteria concerned in the process produce some sort of toxine which so alters the vitality of the tissues surrounding the teeth that any and every mouth organism may assist in the continuation of the process. One of the cultures inoculated was from the mouth of a man suffering from various nervous symptoms, including wasting and loss of power in the legs, which cleared up on attention to mouth hygiene.[1]

Many of the bacteria found in the pus are pathogenic when injected into animals. Thus ten guinea-pigs and five rabbits injected

---

[1] *Trans. Odonto. Soc.*, April, 1902.

subcutaneously with emulsion of pyorrhœa pus in sterile broth, all but one guinea-pig and one rabbit died, in the majority of cases within forty-eight hours.

Some of the animals developed a local abscess at the seat of inoculation which when incised contained a thick viscid pus with an evil smell. Microscopically this pus contained the same morphological forms noted in the coverslip preparations from the original mouth lesion, but the organisms were not obtained in pure cultivation.

The broth cultures showed the same threads of lightly staining bacilli and gave off the same unpleasant smell as do tubes inoculated from the gum pockets.

The organisms found at the *post-mortem* examinations were by no means constant, the organism occurring in the largest number of cases being a bacillus, generally in jointed chains (streptobacilli). This organism was isolated in pure culture from three of the cases and re-inoculated into guinea-pigs, each time producing a fatal result; there was no abscess formation, but the organisms were recovered from the heart blood in pure culture.

From two of the cases Staphylococcus albus was obtained, and in one case B. mesentericus ruber. Several other organisms were also found in various cases, and there is therefore no sufficient data to draw any deductions from beyond the general facts that (*a*) the pus is decidedly pathogenic for animals, and that this pathogenicity is not apparently due to the common pus organisms; (*b*) that the organisms growing in broth cultures are able to elaborate a toxine (apparently by symbiotic activity) which when filtered produces death on inoculation into guinea-pigs. The condition would therefore appear to be primarily toxic, the suppuration with pus formation being a secondary matter.

The pneumococcus was not met with, nor has Bacillus coli appeared on the cultivations except in one case.

In two cases examined a yeast was obtained which caused death when inoculated intraperitoneally into guinea-pigs; the organisms were recovered from the peritoneal cavity in pure culture. Grasset[1] obtained a pathogenic yeast from a mouth abscess, and the one I have isolated appears to be similar.

Troiser and Achlaime[2] also describe a pathogenic yeast obtained

---

[1] *Arch. de Med. Exp. et Anat. Path.*

[2] Fullerton. *Journ. of Path. and Bact.*, 1900.

from the throat of a patient suffering from enteric fever. This organism tended to form hyphæ in culture media, and is interesting in association with the general presence of yeast forms in pyorrhœa pus.

Hunter,[1] in a valuable communication, has called attention to the association of various general disturbances related to local conditions of oral sepsis, and points out conclusively the relation of "septic" gastritis, general septicæmia, and the like, as well as a class of toxic conditions of an ill-defined nature, which owe their origin to uncleanly and suppurative conditions of the buccal cavity.

Hunter ascribes the symptoms to "the pus cocci so frequently present in the mouth," and quotes Miller, Galippe, Vignal and Arkövy. Miller, Netter, and myself, however, agree that the common pus organisms are by no means as frequent in the mouth as would seem probable—in fact only about 10 per cent. of cases examined give cultivations of these organisms. I have already shown, however, in the foregoing experiments that the pus of pyorrhœa, and the products of the activity of the organisms obtained, are extremely pathogenic for animals, thereby confirming Hunter's contentions; and there is no doubt whatever that the swallowing of these organisms and their products greatly influences the health of certain individuals. All persons with septic mouths, however, do not suffer from toxic poisoning, and several of the cases from which decidedly pathogenic results followed the injection of animals with pyorrhœa pus emulsion exhibited no impairment of health in any form, either gastric or general. It therefore becomes an interesting problem that toxic absorption does not always produce the gastric and other effects noted by Hunter.

We have already seen in the chapter on immunity that a large degree of tolerance may be produced in an animal by repeated injections of increasing doses of a given organism or its toxines. I have also referred to Ehrlich's theory of antitoxine formation, and we may I think apply the conception to an explanation of the tolerance to poisons produced in the mouth.

It is well known that individuals suffering from toxic mouth conditions may show no signs of poisoning for long periods, but that often such persons develop symptoms of toxæmia somewhat suddenly, and that once established the recovery is long and tedious.

---

[1] *The Practitioner*, 1901.

Ritchie has recently shown that tetanus toxine if mixed with a proportion of acid does not give rise to tetanic symptoms when injected into a susceptible animal, but a degree of immunity is nevertheless produced.

It is quite possible therefore that some inactivation of the toxine produced occurs, and that a certain amount of immunity is produced thereby, but that under increased dose the immunity breaks down.

CHAPTER XII.

Bacteria only met with in the Mouth.

A NUMBER of bacteria occur with great regularity in all unclean mouths, and wherever any deposit of calculus exists. They exhibit the peculiarity that they will not grow upon ordinary culture media, at any rate when material containing them is inoculated directly from the mouth; they may appear in the abscesses and local inflammations produced in animals, by the inoculation of material taken from the gum margins. Miller was the first to attempt a classification of these mouth organisms, his tabulation being as follows:—

(1) Leptothrix innominata.
(2) Leptothrix buccalis maxima.
(3) Bacillus buccalis maximus.
(4) Spirillum sputugenum.
(5) Spirochæte dentium.
(6) Iodo coccus vaginatus.

Miller found that all these organisms refused to grow upon the ordinary culture media, and in no case was he able to obtain a pure cultivation; occasionally some of the bacteria grew a little, but they soon died out. This question of cultivation is one of the chief difficulties in isolating the above organisms, as not only do they require special media, but they are particularly susceptible to the presence of other bacteria; the mouth streptococcus, in particular, *growing down* almost all other forms.

It is probable that the morphological forms tabulated above will ultimately prove to be related to more than the six species they now represent, but until they have all been obtained in pure culture Miller's tabulation should stand. I have succeeded in cultivating two at least of the organisms in the above list, and it is to be hoped that the others may ultimately be obtained in pure culture. Two other organisms may be added to Miller's list, one the Leptothrix

racemosa of Vicentini, and the Streptothrix buccalis described by myself.

The Group Leptothrix.—A great deal has been written concerning "Leptothrix," and all sorts of curious things said of the doings of the mythical "Leptothrix of tooth decay." Any thread-forming organism has been included under the term, so that not a little confusion exists in the nomenclature; more particularly has this arisen from the fact that no definite rule has been followed, and no proper definition of Leptothrix adhered to.

FIG. 56.—SHOWING VARIOUS FORMS FROM THE MOUTH DIRECT.

The fine threads are Miller's Leptothrix innominate, the thick chain of bacilli B. maximus buccalis.

The term Leptothrix signifies a genus of bacteria belonging to the higher forms of Schizomycetes, and nearly related to the Crenothrix and Beggiatoia.

Zoph, in his classification of bacteria, describes the genus Leptothrix as "spherical, rod-shaped, and filamentous forms, the last showing a difference between the two extremities; spore formation not known, filaments straight or spiral." Migula has proposed as a family name for the various higher bacteria, Chlamydobacteriaceæ, and defines them as "filamentous bacteria composed of rod-shaped

cells and surrounded with a distinct sheath. Division of the cells at right angles to the axis of the filaments. In Phragmidothrix and Crenothrix, gonidia are formed by division in three directions of space. Reproduction by gonidia which are motile or non-motile."

Migula's nomenclature, therefore, is an amplification of the two genera of Zoph, Leptotricheæ and Cladotricheæ.

The divisions of Migula's Chlamydobacteriaceæ are :—

I.—Cells without sulphur granules.

A.—Filaments unbranched.

(i.) Cell division in one direction of space only (*a*) (*Leptothrix*).

(ii.) Cell division before gonidia formation in three directions of space.

(*a*) Filaments with scarcely discernible sheath (*Phragmidiothrix*).

(*b*) Filaments with easily discernible sheath (*Crenothrix*).

B.—Filaments show false branching (*Cladothrix*).

II.—Cell contents contain sulphur granules (*Thiothrix*).

The Beggiatoaceæ are grouped as a separate family.

Zoph includes Crenothrix, Phragmidothrix, Leptothrix and Beggiatoa under the one family Leptotricheæ.

Lehmann and Neumann class Leptothrix, Cladothrix dichotoma (F. Cohn), Beggiatoa, Phragmidothrix and Crenothrix under the term Higher fusion algæ, but disclaim personal knowledge of the group. Several species of Cladothrix and Streptothrix are merged in the genus Actinomyces.

From the above brief review of the literature it will be seen that considerable diversity of opinion exists as to the proper grouping of these higher bacteria; Migula's classification appears to be the one to which least objection can be taken, and is moreover capable of extension, if necessary, to include new forms.

Whatever be the exact definition of Leptothrix decided upon, it is clear that bacilli which are able at times to form threads cannot on that account alone be included as Leptotricheæ; it is, however, impossible to state whether the curious bacillary forms often met with in the mouth are members of the above family or merely involution forms of other bacteria. I am inclined to think that a number of the morphological forms often seen are related to the Blastomycetes, more particularly as cultivations containing these

forms often result in the growth of certain yeasts, many of which produce distinct filamentous forms on some media, but I am unable at present to make any definite statement as to the relationship. The yeast filaments often give the granulose reaction. One organism that is known as Leptothrix epidermidis,[1] produces coiled up and twisted filaments which are slightly motile but show no flagella; the movement is thought to be due to contractility of the organism itself. This organism is said to be common on the skin of man.

It is quite impossible to discuss all the isolated species individually termed "Leptothrix" until fuller knowledge of their biology has been obtained; the species described by various authors are therefore given without any attempt at arrangement, but it is to be hoped that further work will contribute largely to our knowledge of the subject.

The organism described by Vignal as Leptothrix buccalis is supposed to be identical with Leptothrix buccalis of Robin. The organism described by Vignal is a large bacillus, and is the same I have described as B. maximus as far as it is possible to judge from Vignal's observations. The organism does not merit the term Leptothrix, and Miller's term B. maximus is retained (see below).

Leptothrix Placoides alba (Dobrzyniecki, *Cent. für. Bakt.*, B. xxi., 225), obtained only once, from a root filling removed after four years.

Morphology.— Chains of bacilli forming tangled skeins (not branching?); on staining, bacillary and cocci-like bodies are seen in the centre of the threads. The threads are not motile.

Staining Reactions.—Stains by Gram's method, and with the ordinary aniline dyes, best with aniline-gentian violet or fuchsin. Stains blue with acidulated Gram's iodine (granulose reaction).

Biological Characters. — An äerobic, liquefying, non-motile bacillus. Spore formation not observed.

Gelatin Plates.—In forty-eight hours round minute raised white colonies composed of masses of threads resembling the colonies of B. anthracis. In three days the gelatin around the colonies becomes liquefied and the colony floats in the fluid.

Gelatin Streak.—Development slow, in four to five days minute white colonies appear, liquefaction commencing about the tenth day.

[1] Byzozero. *Cent. für. Bakt.*, xx., p. 606., Nos. 16-17.

Agar Plates.—Appearance similar to gelatin plate.

Agar Streak.—A raised clear, cartilaginous mass is formed along the streak composed of isolated colonies having the appearance of placoid scales. In eight to ten days the colonies have become entirely confluent and the whole mass can be removed entire with the platinum needle.

Blood Serum.—Growth similar to agar; no liquefaction occurs.

Potato.—No growth.

Broth.—No growth.

The organism died out before any more observations were made. From the description given of the colonies the organism much resembles the Streptothrix group.

(29) LEPTOTHRIX INNOMINATA (MILLER).

Not yet cultivated and probably including many of the organisms of the mouth of thread-forming habit.

In many mouths, especially those with gingival inflammation, with a considerable deposit of "materia alba" around the teeth, various thread forms are commonly found. Among these bunches of fine slender threads are frequently seen and constitute the L. innominata of Miller (see fig. 56). The threads are long (20 μ and upwards), often curved and twisted, 0·5 μ to 0·8 μ in diameter. They rarely show any division into bacillary elements, but occasionally this may be observed.

The threads generally show unequal staining when carbol-methylene blue is used; some of these threads stain by Gram's methods, others do not. No granulose reaction is given, only a yellow colour appearing.

(30) IODOCOCCUS MAGNUS.[1]

Obtained by Miller from the deposits around teeth on a special medium of "agar-gelatin, dentine glue with 1·5 per cent. of starch and sugar."

No description given beyond the granulose reaction by means of which the organism may be isolated.

(31) LEPTOTHRIX RACEMOSA.

(Vicentini, "Cryptogamic Flora of the Mouth.")

The *Leptothrix racemosa* of Vicentini was at first credited by its discoverer as the origin of all bacteria found in the sputum and saliva,

[1] Miller, "Micro. Mouth," p. 82.

morphological form alone being relied upon. Recently his views have been somewhat modified, but he still considers that many of the morphological forms met with in the mouth are phase forms of this peculiar organism. The organism in question may be found in the majority of mouths, but more plentifully in those mouths where little or no care and attention is bestowed upon the teeth. It may form thick, whitish deposits upon the surfaces of teeth not opposed to one another, and may at times form a thick creamy layer along the gum margin, where it is intermingled with other species of organisms, many of which have been confused with it.

Fig. 57.—Leptothrix racemosa of Vicentini, from Mouth Direct. Balsam mount. Photomicrograph and specimen by Dr. Leon Williams. × 1,000.

In the ordinary method of making coverslip preparations from the mouth the characteristic form of the organism is very apt to be destroyed and the "heads" broken up. Vicentini and Williams,[1] who have worked at this organism, have adopted glycerine as a mountant (figs. 58 and 59), by which method the various forms are more easily preserved intact. Another method which has not been adopted by either of the observers mentioned,

[1] *Dental Cosmos*, April, 1899.

and which gives excellent results, is to examine the organism in the hanging drop, saliva or 0·75 per cent. salt solution being used as the medium. Such preparations are of course not permanent.

The organism, according to Vicentini and Leon Williams, belongs to a higher order than the Bacteria or Schizomycetes, and it is suggested that it should be placed among the Fungi. Williams thinks that the process of sporulation that he has seen is nearly allied to the Uridineæ or Rusts. In coverslip preparations, and particularly in the hanging drop made from the white deposit con-

FIG. 58.—LEPTOTHRIX RACEMOSA OF VICENTINI, FROM MOUTH DIRECT, SHOWING "FRUITFUL HEADS."

Glycerine mount. Photomicrograph and specimen by Dr. Leon Williams. × 1,000.

taining this organism, curious felted masses of entwined threads are seen, many of which appear as if surrounded with closely adhering cocci. Some of these masses are finger-shaped and project from the general mass of the threads and cocci (see fig. 59). After a little search isolated specimens can be found, when the cocci-like bodies are seen to be arranged in regular order and, according to Williams and Vicentini, attached to the thread by basidia which, according to the former, are seen to pass from the thread to the "spore." The central thread can easily be traced through the mass of spore-like

bodies to its free end, the whole appearance reminding one of the common "Friar's Cowl" of the hedge-rows when ripe. Williams gives a number of photographs of this organism, some of which show the cocci-like bodies particularly well, but it is by no means certain that these basidia are not artifact. If the "spores" are attached with these basidia or short stalk-like processes of the central thread, one would expect to observe the basidia on free spores, or on parts of the thread, which so far has not been accomplished. If, on the other hand, the spores are attached one to another they are more referable to the type of some of the moulds (*cf.* Penicillium).

FIG. 59.—FRUITFUL HEADS OF L. RACEMOSA, SHOWING ARRANGEMENT OF GONIDIA.
Photomicrograph and specimen by Dr. Leon Williams. × 2,000.

The threads of this peculiar organism have been shown by Williams to stain in a special way with a modification of the Gram method, as follows:—the material containing the threads, &c., is carefully made into an emulsion with distilled water and then stained with hot aniline gentian violet for from eight to ten minutes. The specimen is then placed in hot iodine solution to fix, washed in absolute alcohol and then counterstained with methylene blue;

by this method of staining, spore-like bodies are seen to occupy the ends of the segments of the threads. The spore-like areas may be stained in another way, which rather precludes their description as spores. A coverslip preparation is made and MacConkey's capsule stain[1] poured on. The preparation is then warmed until steam commences to be given off (it must not be allowed to boil), the stain is left on the coverslip for five minutes, washed off and the coverslip mounted. The curious beaded appearance of the threads is

FIG. 60.—LEPTOTHRIX RACEMOSA, MOUTH DIRECT. STAINED CAPSULE STAIN. Showing darkly staining dots. × 1000.

brought out by this method; the stained "spores" are more to be regarded as arthrospores rather than true endogenous spores. On staining with Miller's iodine or with iodine acidulated with sulphuric acid, some of the areas apparently corresponding to the areas that stain with the foregoing method take a faint blue or violet tinge—in other words, they give the granulose reaction.

Good specimens of this organism are difficult to obtain, and great care must be exercised in making the coverslip preparation.

[1] Dahlia, ·5 gm., methyl green (00 crystals), 1·5 gm., sat. alcoholic fuchsin, 10 cc., water to 200 cc.

The best method to adopt is to suspend some of the material containing the organism in distilled water. A large drop is transferred to a coverslip and allowed to dry—anything like spreading should be avoided. Flaming the coverslip is also liable to break up the organism, and it is best to fix with alcohol and ether as in staining blood-films.

When Vicentini first sent the description of the organism to Miller the latter was of the opinion that it should be classified as a Cladothrix or Crenothrix rather than a Leptothrix, but upon the representation of Vicentini he withdrew his objection. Williams, while accepting the term Leptothrix provisionally, has shown that the "fruitful heads" may be not inaptly compared to the fructification of the Cordiceps militarius and Botritis Bassini,[1] providing the sterigmata and basidia exist. At the same time the method of sporulation or fructification of the Cladothrix and Crenothrix have some points in common with the organism under discussion.

The gonidia or asexual spores of Botritis Bassini are supported upon well-marked sterigmata or basidia, the term basidium being used in both a general sense when it is applied to the end of the thread that undergoes asexual sporulation, and in a special sense, when it is used to indicate the stalk upon which the asexual spore or gonidium is carried and by which it is attached to the parent thread.

In Penicillium the carpophore or special spore-bearing hypha is an erect branch of the mycelium, the terminal portion of which divides into numerous branchlets which in turn divide up into a chain of naked gonidia without special sterigmata, a condition with a little modification that is not unlike the sporulation of the L. racemosa.

Again, the Crenothrix breaks up into a multitude of spore-like bodies, the terminal portion of the thread undergoing multipartate division into gonidia; these may be extruded or remain attached to the interior of the thread. It is also to be noted that if a freely growing coccus, such as Staphylococcus albus, be grown in the presence of an equally freely growing bacillus, coverslip preparations made from the mixed culture will show cocci apparently attached to the thread forms and to the shorter bacilli as well, much in the same way as the preparation from the mouth shows cocci attached

[1] Du Bary, "Morphology of Fungi," p 65.

threads supposed to be the early sporulation of the L. racemosa. It is, moreover, not difficult to produce the appearance of sterigmata by using a stain like gentian violet, which is notorious for its quick deposit.

There are many other points of similarity between the L. racemosa and some of the Ascomycetes, for further particulars of which the reader is referred to Du Bary's book.

As the organism we have been discussing has as yet defied attempts at cultivation, it is difficult to assign it to any particular genus. Its morphology does not so far conform with any one class of Ascomycetes or Schizomycetes.

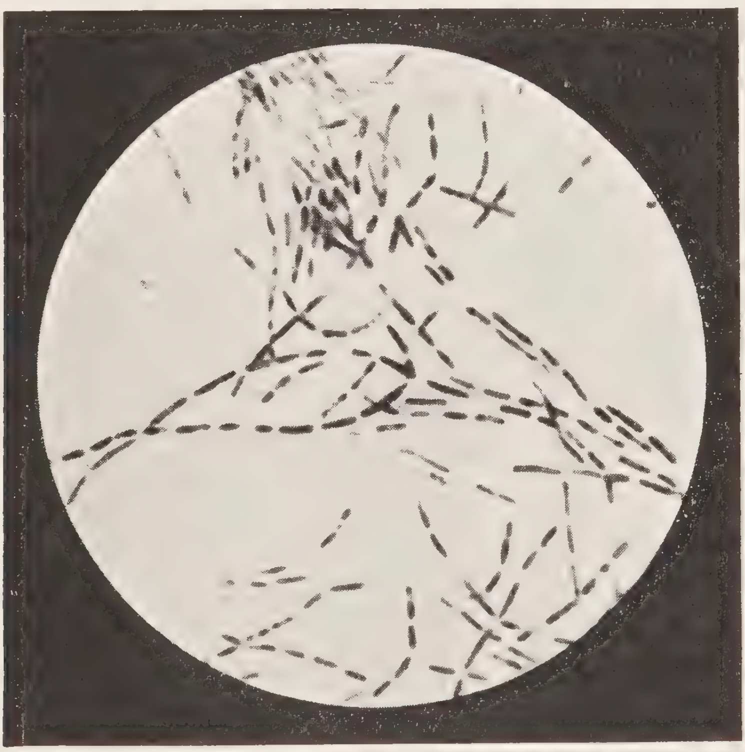

FIG. 61.—BACILLUS MAXIMUS.
From agar cultivation twenty-four hours old at 37·5° C. Stained Gram. × 1,000.

(32) BACILLUS MAXIMUS.

Leptothrix buccalis (Vignal). *Bacillus maximus*, *Leptothrix buccalis maxima* (Miller).

The largest of the mouth bacteria occurs in most dirty mouths and is often seen associated with the "Leptothrix" innominata.

Miller, who first applied the term B. maximus, describes another

organism, Leptothrix buccalis maxima, which differs in two particulars only from B. maximus—the threads do not give the granulose reaction and the segments are of greater length. Neither of these organisms were obtained in pure culture, and the differentiation is therefore not sufficient to class them as of different species.

Vignal's Leptothrix buccalis is a bacillus, and in its cultural characters corresponds closely to those of the large bacillus I have obtained in pure culture from the mouth (see fig. 61). Under these circumstances Miller's term Bacillus maximus is adopted.

Under favourable circumstances this organism may be obtained in pure culture by making an emulsion in broth of the material containing the bacillus and other mouth bacteria; the emulsion is then plated on a series of (1) maltose agar, or (2) potato gelatin plates and the colonies carefully examined for the organism sought for. The bacillus requires some time before it becomes acclimatised to the conditions of artificial media, but when it has developed its "laboratory habit" it grows freely.

Although the organism forms endogenous spores I have never obtained cultivations from the mouth direct by the method of differential sterilization (see p. 19).

Morphology.—Thick jointed threads 0·5 to 1·5 μ broad, 10 to 20 μ long; some threads may be much longer, and occasionally twisted, especially upon old potato cultures. The individual elements are 1 to 4 μ long but may be much longer.

From the mouth direct and in the cultures the bacilli show considerable irregularity of the protoplasm which is brought out on staining. The "spotted" appearance of the bacilli when stained by carbol fuchsin or carbol methylene blue is due to a fragmentation of the cytoplasm. Staining by hot carbolic fuchsin shows spores situated at the ends of the threads, while large, clear unstained areas may be met with which appear to be spaces left by the extruded spores, and also to the effects of plasmolysis.

Staining Reactions.—Stains by Gram's method, and by the ordinary aniline dyes. With carbol-methylene blue red granules often appear in the older threads. With MacConkey's capsule stain isolated masses of deep staining occur in the interior of the threads. With Möller's method well marked dark red spores are found. By staining with Pitfield's method a few lateral flagella are to be found; they are not present in great numbers, but may be as many as six lateral and one terminal. On the threads only a few lateral

flagella on isolated segments are found. A few of the threads give the granulose reaction. Large oval involution forms appear in old cultures.

Biological Characters.—An äerobic, facultative anäerobic, liquefying motile bacillus. Forms spores.

Gelatin Plates, 22° C.—At the end of twelve hours white, grey, flat, round colonies with entire edge. The central area is darker.

Microscopically, $\frac{2}{3}$ obj.—Edge irregularly dentate, yellow; centre dark brown, irregular, the whole finely fibrillated (marmorated).

Gelatin Stab, 22° C.—Liquefaction in twenty-four hours with cone-shaped depression (infundibuliform). Growth occurs along the line of stab below the liquefaction. The apex of the cone is occupied by a thick flocculent precipitate. A slight flocculent scum appears upon the surface, and numerous flocculi appear in the fluid.

Gelatin Streak, 22° C.—Well marked groove of liquefaction generally in twenty-four hours. The fluid is filled with flocculi.

Agar Plates, 37·5° C.—Brownish, round, raised colonies, similar to gelatin plate.

Agar Streak.—Brownish-grey streak with irregular, flocculent edge. The surface is distinctly granular, especially when seen through a small lens, and has the appearance of frosted glass. It does not become corrugated. The film is easily removed with the spatula, and does not become dry as does B. subtilis. The bacilli form long articulated threads with well marked spores.

Blood Serum, 37·5° C.—Twelve hours: raised, moist, grey, edge much less irregular than on agar. No liquefaction occurs.

Potato, 37·5° C.—Twenty-four hours: well marked, grey, moist growth limited to the inoculated area, and showing no tendency to spread. Later the growth becomes a dirty grey.

Broth, 37·5° C.—Twelve hours: flocculi white to dirty grey, which fall and produce a thick flocculent precipitate; very little general turbidity. A flocculent pellicle may form at a later stage. The bacilli are motile, but not excessively so. No indol reaction.

Litmus Milk.—Well marked acid reaction, no clot is formed.

Glucose, Lactose, Maltose Broth.—Well marked acid reaction in two days. No gas is given off.

No gas formed on gelatin shakes. Grows anäerobically on glucose formate media, but not so well as äerobically. No gas on glucose formate broth.

Pathogenesis.—Undetermined.

Since the first description of this organism in 1898 I have obtained similar organisms from twelve cases.

VIBRIOS OF THE MOUTH.

Considerable interest is attached to the fact that comma-shaped and spiral bacteria are present in a large number of mouths, and that in certain conditions of pathological importance, particularly ulcerative stomatitis, vibrios are present in great quantities, so much so, in fact, that Bernheim[1] considers them the active agents. They are always present during subacute inflammatory conditions of the mucous membrane of the mouth; are frequently met with associated with B. diphtheriæ upon the tonsil—in fact in several cases I found them alone with the Klebs-Loeffler bacillus. They are also found in the cavities of tubercular lung (Artrault),[2] and are not uncommonly found in antral suppuration, where I have met with them in seven cases. Spirilla, having the same characteristics in that they are excessively difficult to obtain in pure culture, are frequently present in fæces, and in one case of Bright's disease spirilla were present in the peritoneal fluid.

Miller, in 1883, first called attention to the presence of the comma-shaped bacilli in the mouth, and a year later Lewis described organisms resembling the cholera vibrio in the oral secretions of healthy persons.

The researches of Miller carried on for two or three years with a very large variety of culture media did not result in the isolation of a pure cultivation of these spirilla. Colonies did occasionally develop, but never withstood the transference to a second tube of media. At the same time Miller obtained cultivations of several species of spirilla, two of which he discusses.

The one proved to be the Vibrio Finkler-Prior, the other was a non-motile organism producing curious twisted and contorted forms, some resembling the capital letter O. The old cultures formed streptococci like involution forms. It appears evident from Miller's work, and from my own experience, that the spiral and comma-shaped bacteria of the mouth do not all belong to the same species; although in all "dirty" mouths (*i.e.*, with deposits of calculus or

---

[1] *Semaine Medicale*, 1896.

[2] *Arch. de Parasitologie*, Tome i., No. 2.

FIG. 62.—SPIRILLUM SPUTUGENUM (Spirilla).
From mouth direct ; gentian violet stain. x 1,0C0.

FIG. 63.—SPIRILLUM SPUTUGENUM (Comma forms).
From mouth direct ; gentian violet stain. x 1,000.

"materia alba"), spiral or comma-shaped forms are constantly found. These spirilla are extremely difficult to obtain in pure culture, and it was only after two years of experiment, during which forty different species of media were tried, that a culture was obtained. In many cases the organisms grew for a short time, but died as soon as they were transferred to another medium.

The mouth spirillum generally grows for a short time upon blood serum, and may also be observed upon several fluid media but only in small numbers. Thus on beer wort, mucin broth (made from snails), saliva filtered and 1 per cent. peptone added, maltose broth with 0·05 per cent. of potassium sulphocyanide, egg broth and alkali albumin broth, all will occasionally show a limited development. The two media which I have found to give the best results are saliva set with agar, and potato gelatin; the latter medium is not a favourable one for the mouth streptococcus, which otherwise grows to the exclusion of most other forms.

The method of obtaining the spirilla is as follows: successive streaks are made upon a number of tubes of the potato medium, and in three or four days a second series of tubes is streaked with any minute pin's point colonies which show spirilla. The second series may often require to be treated as the first, and even then the organisms have a great tendency to die out.

When a culture is obtained it requires subculturing every few days for two or three weeks, after which it gradually becomes accustomed to the altered conditions and develops fairly well. The early cultures do not form typical spirilla; under the hanging drop, however, the characteristic movements are seen.

(33) SPIRILLUM SPUTUGENUM.

Morphology.—Vibrio, occurring in young cultures as comma-shaped rods 0·1 to 0·3 μ in breadth, 1 to 2·5 μ long, with rounded or pointed ends. In old cultures well marked spirilla are formed, some composed of commas united in series, others of spirilla with three or four turns without a break. Very long threads are also met with; these are often thinner and irregular (spirochæte). Spiral forms best marked on both cultures in forty-eight hours. No endogenous spores were found, but irregular spherical bodies are found attached to the older threads (see fig. 66) as well as independently (? arthrospores).

Staining Reactions.—Does not stain by Gram's method, best

with carbol fuchsin, or carbol gentian violet, or aniline gentian violet. The organism only stains faintly with carbol methylene blue. In old cultures the threads stain unequally, and give the appearance of chains of bacilli with unstained intervals. With Pitfield's method single terminal flagella are seen.

Biological Characters.—An äerobic facultative anäerobic, liquefying motile spirillum. Does not form spores (endogenous). Arthrospores formed (?).

Gelatin Plates, 22° C.—At the end of forty-eight hours minute greyish-white colonies, much like streptococci, appear, they are moist and flat, and the gelatin around them soon commences to liquefy.

FIG. 64.—SPIRILLUM SPUTUGENUM, FRESHLY ISOLATED FROM THE MOUTH. (Spirillum form not yet well developed.) × 1,000.

Microscopically, $\frac{2}{3}$ obj.—Brownish, round, or oval, not granular, with darker opaque irregular centre.

Gelatin Stab, 22° C.—Cup-shaped liquefaction (napiform), in four days, little fluid; the tube may often be inverted without any of the contents escaping; white flocculi appear in the fluid and a considerable deposit at the bottom.

Gelatin Streak, 22° C.—Groove of liquefaction in three days with white flocculi in fluid. No pigment is produced.

Agar Plates, 37·5° C.—Brownish, flat, smooth, moist, central portion elevated, edge entire or gyrate.

Agar Streak, 37·5° C.—Good growth in twenty-four hours with defined, entire edge slightly raised, grey, translucent. Later the growth becomes buff coloured.

Blood Serum, 37·5° C.—Grey, smooth, moist streak. The medium is slowly liquefied.

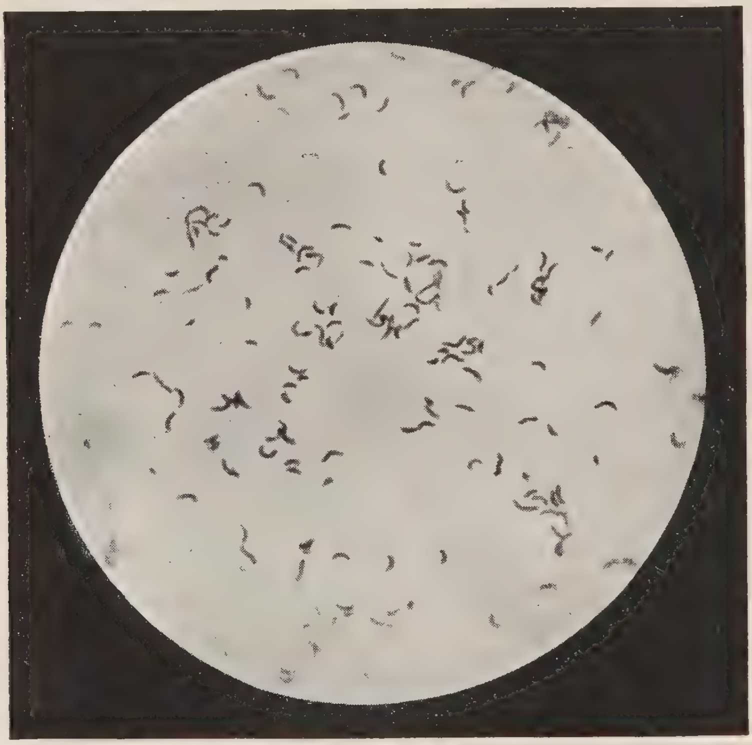

FIG. 65.—SPIRILLUM SPUTUGENUM.

Agar cultivation at twenty-four hours (comma forms). Stained gentian violet. × 1,000.

Litmus Milk, 37·5° C.—Well marked acid reaction in twenty-four hours, with coagulation of casein in five days. Not re-dissolved.

Broth, 37·5° C.—In twenty-four hours general turbidity with slight pellicle. A four days' culture gives a well marked cholera-red reaction (nitroso-indol), with nitrite free sulphuric acid; H_2S present.

Potato.—No apparent growth in twenty-four hours at 37·5° C.; two to three days at 22° C. well marked rich red-brown colouration, moist and shiny. Involution forms and threads common.

Glucose, Lactose, Maltose Broth.—Well marked acid production in forty-eight hours; gas evolved.

Anäerobiosis.—Grows on glucose formate media without oxygen in Buchner tubes, and produces gas on glucose formate broth.

Pathogenesis.—Pathogenic for guinea-pigs (four only inoculated), 1 cc. of agar culture emulsion fatal in three days when injected into peritoneal cavity.

FIG. 66.—SPIRILLUM SPUTUGENUM.

Broth cultivation at seven days, showing involution forms (? arthrospores). × 1,000.

(34) SPIROCHÆTE DENTIUM.

Probably identical with foregoing. Occurs in fine irregular threads, 0·1 μ wide, 5 to 7 μ long. Sometimes seen with coccus-like bodies attached to thread (? arthrospores). Found in deposits along gum margins. Stains with difficulty with carbol methylene blue and not by Gram's method; so far I have not been successful in observing flagella.

The twists or turns of the organism are more angular than those of the ordinary spirillum, and the motility appears confined to quiet revolution upon their long axis.

THE GENUS STREPTOTHRIX.

A good deal of interest has been awakened with regard to this group since the diphtheria and tubercle bacilli were shown by Hueppe and his pupil Fischel, as well as the earlier work of Roux, Nocard, and Metchnikoff, to occasionally exhibit distinctly branched forms.

Still later Nocard, Eppinger, Moeller, and many others have shown that some branching fungi of the actinomyces class are "acid-fast," that is to say, they resist decolourization with weak (25 per cent.) solutions of mineral acids when stained with hot carbolic fuchsin, just as do the tubercle, leprosy and smegma bacilli. Portions of the threads are under these circumstances almost indistinguishable from the tubercle bacillus, especially when the examination has to be made in sputum, as cited in the case described recently by Birt and Leishman.[1]

Mycologists differ somewhat as to the proper nomenclature of the group of fungi of which the "ray fungus" or "actinomyces" is the type. The majority of English and American pathologists adopt the term Streptothrix for the group comprising the organisms which have been variously termed, Actinomyces Cladothrix, Oospora, and Nocardia. Lehmann and Neumann class the genus as Actinomyces.

For the present at any rate Streptothrix is perhaps the better term, and is adopted here. The characteristics of the genus are briefly :—delicate threaded organisms free of chlorophyl, showing a true branching mycelium; portions of the threads show club-shaped endings and gonidia which may also be developed in the mycelium. Fragmentation of the mycelium occurs with the production of various morphological forms not to be differentiated from the various forms of Schizomycetes. The mycelium may be developed from the gonidia or from the fragmented threads.

S. actinomyces has already been described (p. 124).

A short time since I described (*Trans. Odont. Soc.*, June 18, 1899) an organism isolated from the mouth which resembled the Cladothrix dichotoma of Cohn, as far as I was then able to judge, from the Cladothrix dichotoma in the Guy's Laboratory. The organism in question is, however, to be placed in the same category as the actinomyces bovis, and conforms to the generic description of Streptothrix given above; it should therefore be termed Streptothrix buccalis.

---

[1] *Journal of Hygiene*, Ap., 1902, p. 120.

Since the first description of that organism was published I have met with it upon many occasions in the mouth, particularly in the thick viscid pus sometimes emanating from the gum pockets around the teeth affected by pyorrhœa alveolaris. On one occasion it appeared in the heart blood of a rabbit dying from the injection of pyorrhœa pus. The organism obtained from this source conforms to the general characteristics of the other specimens obtained by cultivation. I have not met with it, so far, in normal mouths. There is therefore some reason to suppose that it may have a relationship to alveolar pyorrhœa.

(35) STREPTOTHRIX BUCCALIS.

Obtained from pyorrhœa pus, and from the white deposits around the teeth, also in gingival inflammation.

Morphology.—Filamentous forms; in young cultures a tangled mycelium is produced, the threads of which show well marked lateral branches; the ends of those threads are frequently club-shaped. Dichotomous branching or branching by longitudinal fission of terminal filaments not observed. The lateral branches are unequally distributed upon the thread and often present a constriction at their junction with the main stem. The terminal portions of the threads may taper to a point or show distinct enlargement (fig. 67).

Somewhat later the threads and clubbed extremities undergo changes resulting in the thread staining unequally, especially with gentian violet or Gram's method; at the same time segmentation transverse to the long axis results in the production of bodies which may be termed gonidia. Fragmentation now occurs, the mycelium splitting up into a series of forms morphologically simulating various bacteria (fig. 69), and as some of the threads are slightly spiral, comma shapes and spirilla are also formed.

The gonidia set free germinate and produce threads by lateral extension, and thus the cycle is complete. On solid media particularly the colonies become covered with a white powder consisting of the gonidia which, if transferred to another tube, grow into colonies.

Staining Reactions. — Stains by Gram's method and by the ordinary aniline dyes. The clubs take the stain most deeply. Not acid-fast to carbol fuchsin and 25 per cent. sulphuric acid.

Fig. 67.—Streptothrix buccalis from Mouth Direct.
Stained Gram, showing branched threads and clubs. × 1,000.

Fig. 68.—Streptothrix Buccalis.

Forty-eight hours' cultivation on agar stained Gram. Showing branched threads. × 600.

Biological Characters.—An äerobic, liquefying streptothrix.

Gelatin Plates, 22° C.—At the end of three to four days (rarely earlier) minute, hard, raised, colourless and spherical colonies appear, which increase somewhat rapidly and occasionally form cone-shaped projecting points the tops of which become coated with a white powder. Liquefaction commences somewhat later and the colonies gradually sink into the fluid gelatin. Occasionally the colonies do not produce the projecting cone-shaped points but remain flat and ring-shaped, the centre remaining clear.

FIG. 69.—STREPTOTHRIX BUCCALIS.

Seven days' cultivation on potato stained Gram, showing involution forms due to fragmentation of the threads. × 1,000.

Gelatin Stab, 22° C.—Slight development occurs along the line of inoculation in minute beaded colonies. Liquefaction commences at the surface about the fifth day and extends to the tube walls; the liquefied medium is separated from the solid by a horizontal plane (stratiform).

Gelatin Shake, 22° C.—No gas bubbles are produced, and a faint cloud of minute colonies, best marked at the surface, appears in four to five days. Liquefaction stratiform.

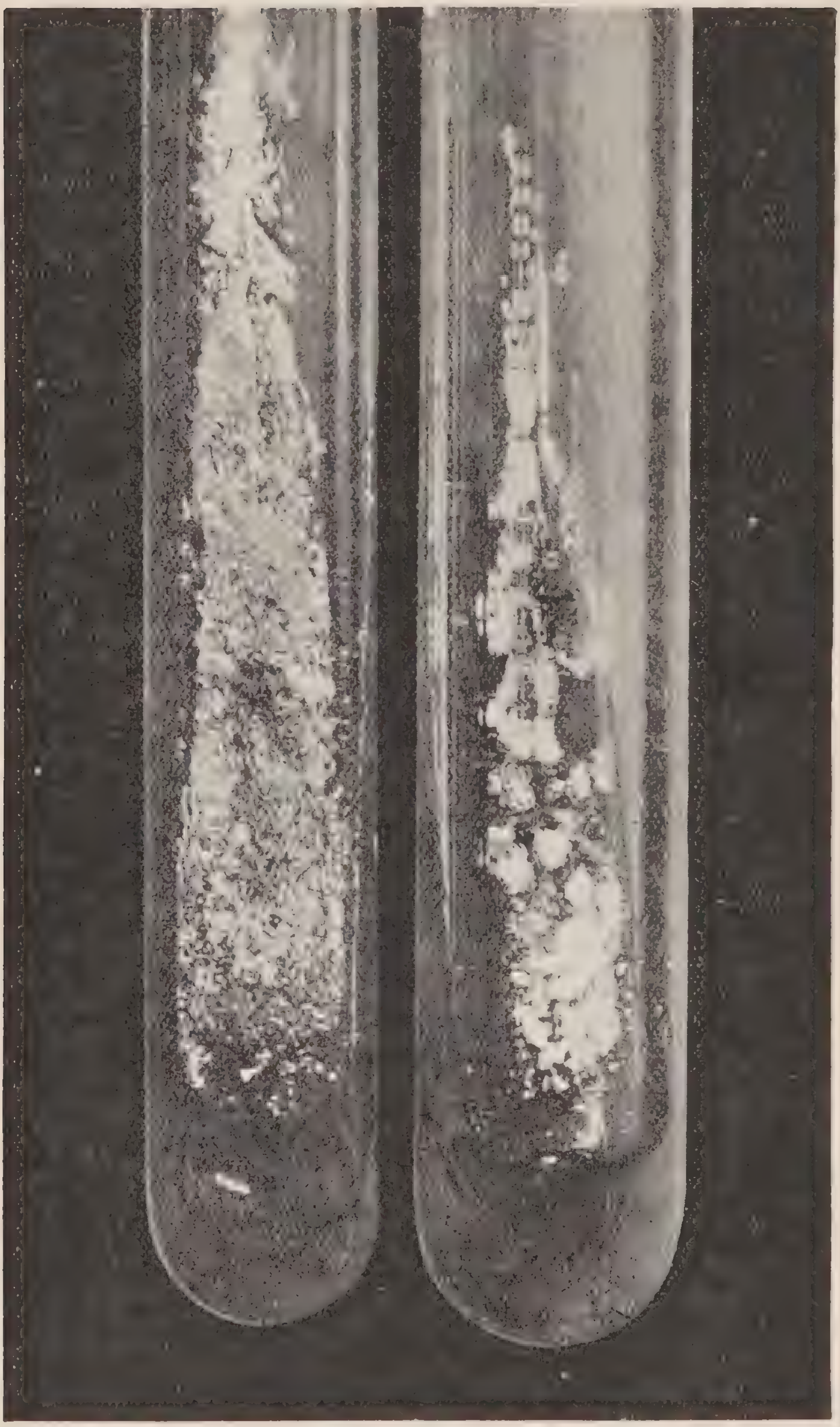

FIG. 70.—STREPTOTHRIX BUCCALIS.
Five days' cultivation in agar.

Agar Plates, 37·5° C.—In twenty-four hours small, hard, convex colonies, having a cartilaginous consistency; in three or four days they become truncated cones, and later surmounted with white powder composed of gonidia. The cones often crack across the summit. The colonies eventually produce a slight depression in the agar; they are extremely difficult to remove with the platinum needle, and to make a satisfactory coverslip preparation it is best to crush a colony between two cover glasses.

Blood Serum, 37·5° C.—No discolouration on this or any other media, with the exception of an occasional colony in freshly isolated specimens. Liquefaction occurs.

Litmus Milk, 37·5° C.—In two days no change, later precipitation of casein occurs, the milk clearing and taking on a bluish-purple tinge. Morphologically, mostly streptobacilli.

Broth, 37·5° C.—Flaky particulate scum gradually developing into spherical colonies which fall to the bottom. A layer is generally retained around the meniscus, which becomes chalky white.

Potato, 37·5° C.—Yellowish or orange-brown discolouration, not constant, with colonies flat or raised and about the size of small shot. A chalky-white appearance is later caused by the formation of gonidia.

Spore Formation.—No endogenous spores observed; cultures killed by exposure to 75° C. for ten minutes. Motility not present. No sulphur grains seen.

All the cultures give off a characteristic musty smell like a damp cellar.

CHAPTER XIII.

Saprophytic Bacteria of the Mouth not Described in Previous Sections.

(36) BACILLUS COLI COMMUNE.

FOUND extremely widely distributed, in water, in fæces, in certain diseases of animals and in diseases of men. Commonly present in milk, &c. Often present in the mouth. Frequently present in abscesses situated near digestive tract.

Morphology.—Bacilli 1 to 4 μ long, and 0·4 to 0·6 μ broad. Often forms rods of considerable length. The ends are rounded. Actively motile in young cultures.

Staining Reactions.—Stains easily with the usual methods, but not by Gram's method. In old cultures polar staining occurs. The flagella may be stained by Pitfield's or van Ermengem's methods; they are generally twelve, rarely more than fourteen, arranged around the bacilli (peritrichic).

Biological Characters.—An äerobic, facultative anäerobic, non-liquefying motile bacillus. No spore formation. Grows well at 37° C. and at the ordinary room temperature (22° C.).

Gelatin Plates, 22° C. *Superficial Colonies.*—At first small yellowish punctiform colonies, later becoming large irregular; edge lobulate or dentate, shining. Centre opaque and white and often a little raised.

Deep Colonies.—Punctiform, later wheatstone-shaped, yellowish. No liquefaction.

Microscopically.—Irregularly marked surface, showing faint, irregular stripes or traversed by vein-like markings as in marble (marmorated).

Gelatin Stab, 22° C.—Well marked growth to bottom of stab, whitish-grey, granular; no liquefaction. Surface thin, whitish-green, flat, edge notched.

Gelatin Shake, 22° C.—Three days: well marked cloud of gas bubbles; no liquefaction.

Gelatin Streak, 22° C.—Spreading, white, thin, granular, as on surface of stab.

Agar Streak, 37·5° C.—Grey-white, glistening, moist, translucent. Condensation water clear with slight precipitate.

Potato, 22° C.—Yellow to yellow-brown discolouration of potato occasionally occurs.

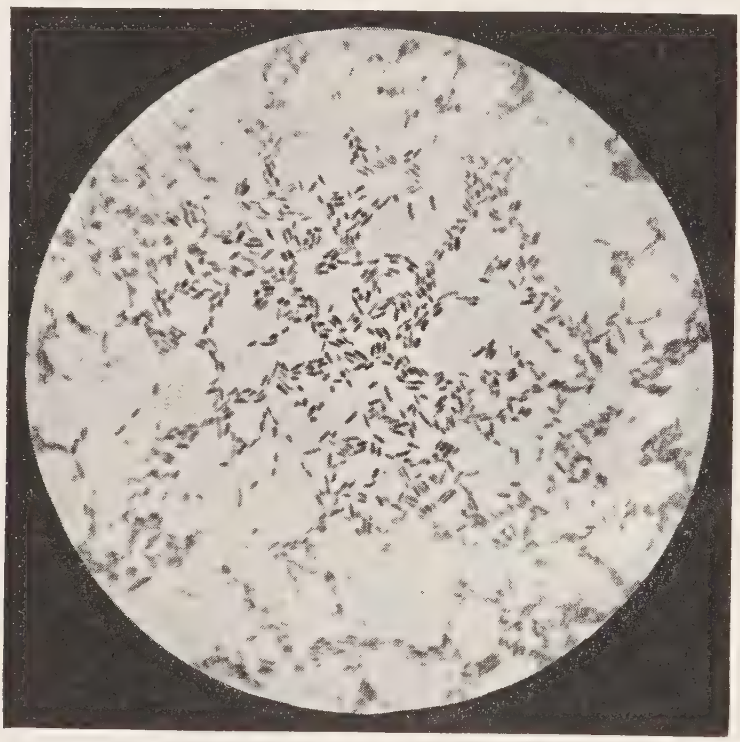

FIG. 71.—BACILLUS COLI COMMUNE.
Agar cultivation twenty-four hours old. × 1,000.

Litmus Milk, 37·5° C.—Coagulation and marked acid reaction. H_2S and indol produced.

Broth, 37·5° C.—Dense turbidity with thick sediment. Indol in seven days or less.

Lactose, Maltose, Glucose Broth.—Acid fermentation and gas given off (CO_2).

Anäerobiosis.—Grows well on glucose formate media, producing much gas on glucose formate broth.

Pathogenesis.—Variable, some cultures producing death in one to five days, with general septicæmia when injected intraperitoneally,

Subcutaneous injection may produce local necrosis of skin. Appears in blood stream a few hours before death in some diseases, and often present in the blood three or four hours after death.

(37) BACILLUS LUTEUS.

(Dobrzyniecki, *Cent. für Bak.*, Bd. xxi., p. 835.)

Found in dental caries.

Morphology.—Bacilli 1·5 μ long, irregular in size, non-motile. Stains by Gram's method.

Gelatin Plates.—Minute punctiform colonies in two days; later the colonies become larger, round, well defined, and golden-yellow in colour.

Gelatin Stab.—Development occurs to the bottom of the stab and is a light yellow colour below and golden-yellow above. The gelatin is not liquefied.

Agar Streak.—In forty-eight hours a golden-yellow moist streak is formed, darker in the centre than the edge.

Potato.—Similar to agar; no colouration of the medium occurs.

Pathogenesis.—A slight local reaction produced in mice and guinea-pigs, but no general reaction.

(38) BACILLUS BUCCALIS MINUTUS.

Synonym.—Bacillus g, Vignal.

Found by Vignal in the salivary secretions of healthy persons.

Morphology.—A very short bacillus, with round ends, almost as broad as long; in cultures upon agar the length is from 0·5 to 1 μ, usually about 0·7 μ; in neutral bouillon it is from 1 to 1·7 μ long. In old cultures involution forms are common; in stained preparations the two ends are more deeply stained than the central portion.

Biological Characters.—An äerobic, liquefying, chromogenic bacillus. Produces a yellow pigment. Spore formation not observed. Motility not mentioned. Grows slowly at the room temperature.

Gelatin Plates.—At the end of forty-eight hours the colonies are round, with refractive contour and of a mastic-yellow colour; they are but slightly elevated, and the gelatin commences to liquefy around them.

Gelatin Stick Cultures.—At the end of forty-eight hours a yellowish-white growth is seen along the line of puncture, and upon the surface a layer having the same colour and several millimetres in

diameter has developed; by the fourth day the surface growth has increased to twice the size and is yellow at the centre, while the periphery is white. The growth along the line of puncture is abundant, and consists of small, closely crowded colonies; below the surface growth a cup-shaped cavity filled with clouded liquefied gelatin is seen. By the sixth day a small funnel of liquefaction has formed, the liquefied gelatin is clear, and contains some white flocculi in suspension. By the twelfth day the gelatin in the tube is completely liquefied, an abundant yellow deposit is seen at the bottom, and the liquefied gelatin has the same colour.

Surface of Agar.—Golden-yellow plaques are developed, which are easily removed with the platinum needle.

Bouillon.—A thin, iridescent pellicle is formed upon the surface, and the fluid below is clouded, while an abundant yellow deposit accumulates at the bottom. Does not grow well in acid bouillon.

Potato.—At the end of forty-eight hours a thin and extended layer is formed of a yellow colour, which later has a brownish tint.

(39) BACILLUS BUCCALIS FORTUITUS.

Synonym.—Bacillus j, Vignal.

Found by Vignal in the salivary secretions of healthy persons.

Morphology.—Bacilli with square ends, from 1·4 to 3 μ long; often united in pairs, the elements of which may be joined at an angle of greater or less degree.

Biological Characters.—An äerobic, liquefying bacillus. Spore formation not observed. Motility not mentioned. Grows at the room temperature in the usual culture media.

Gelatin Plates.—At the end of forty-eight hours, small round colonies are developed, which increase considerably in thickness and diameter, and by the fourth or fifth day have caused liquefaction of the surrounding gelatin.

Gelatin Stick Cultures.—At the end of forty-eight hours a small mass has formed at the point of inoculation, and a scanty line of development is seen along the track of the inoculation needle; by the fourth day the growth has extended over the entire surface and presents a decided prominence at the centre. The gelatin below is liquefied, and remains transparent with some opaque white flocculi in suspension; by the twelfth day the liquefaction extends to a depth of 2 cm., and a yellowish-white, abundant deposit is seen at the bottom.

Surface of Agar at 36° to 38° C.—Small, white, opaque colonies are developed which present a small, nipple-like projection at the centre.

Bouillon.—A diffuse cloudiness is produced; a thick, dull white layer forms upon the surface, and an abundant dull white deposit is seen at the bottom of the tube. Does not grow well in acid bouillon.

Potato.—A rather thick growth is developed, which extends slowly and acquires a slightly pinkish tint.

(40) BACILLUS B OF VIGNAL.

Obtained by Vignal in cultures from healthy buccal secretions.

Morphology.—Bacilli with square ends, straight or slightly curved, about 0·5 μ in diameter and varying greatly in length, from 1·5 to 6·5 μ; often united in chains.

Biological Characters.—An äerobic, liquefying bacillus. Spore formation not observed. Motility not mentioned. Grows rather slowly at the room temperature, more abundantly at 37° C.

Gelatin Plates.—At the end of twenty-four hours, at a room temperature of 18° to 20° C., prominent, small, grayish-white colonies are developed upon the surface. At the end of forty-eight hours a collarette with irregular festooned margins is developed around this central mass; this is thinner and much more transparent than the central portion of the colony. Under a low power it is seen to be formed of an innumerable series of skein-like bundles, arranged side by side and more or less twisted, which proceed from the central mass.

Gelatin Stick Cultures.—At the end of forty-eight hours, a small, flat mass is developed at the point of puncture, and a scanty growth is seen along the line of inoculation.

On the fourth day the superficial growth covers the entire surface, it is translucent by transmitted light, and white by reflected light. By the sixth day the gelatin is liquefied to a depth of 1 cm. below the superficial growth; the liquefied gelatin remains transparent; upon the surface is seen a white, membranous layer, and at the bottom a rather scanty white deposit; liquefaction slowly extends downwards, and by the twelfth day has reached a level corresponding with the bottom of the line of puncture.

Surface of Agar.—At the end of twenty-four hours at 36° to 38° C., a dull white layer, having a thickness of about 1 mm., is developed; this is easily broken up with the platinum needle.

Bouillon.—A slight cloudiness is quickly produced, a thin film forms upon the surface, and a scanty white precipitate at the bottom of the tube.

Potato.—At the end of forty-eight hours a layer the size of a five-franc piece is developed, which has a pale pink colour and a rough surface—resembling a lichen.

Blood Serum.—Is liquefied rather rapidly, and acquires a brownish colour, while an abundant white precipitate accumulates at the bottom of the tube.

(41) BACILLUS G OF VIGNAL.

Found by Vignal in the salivary secretions of healthy persons.

Morphology.—Bacilli with slightly rounded ends from 0·8 to 1·2 μ in length when cultivated upon agar, and from 1·4 to 2·4 μ when cultivated in neutral bouillon; usually solitary, occasionally united in short chains.

Biological Characters.—An äerobic, liquefying bacillus. Spore formation not observed. Motility not mentioned. Grows rather slowly at the room temperature—more rapidly at 37·5° C.

Gelatin Plates.—At the end of forty-eight hours, small, projecting, opaque, white colonies are developed; at the end of four days the colonies are seen as conical, opaque, white masses divided into about twenty segments by grooves which start from the summit.

Gelatin Stick Cultures.—A small but prominent white mass is seen at the point of puncture, and a scanty line of development along the track of the inoculating needle; on the fourth day the surface growth has extended nearly to the walls of the tube, and just below this some fine branches are given off from the line of the growth; the sixth day the entire surface is covered, and the gelatin below is liquefied for a short distance; the eighth day the liquefaction has extended downward, and the solid gelatin below has a clouded appearance owing to the development of a quantity of small white colonies; by the twelfth day the liquefied gelatin has a depth of about 2 cm., a shining, white mycoderma is seen upon the surface, a white deposit at the bottom, and below this numerous small colonies in the solid gelatin.

Surface of Agar.—Very adherent, white colonies are formed, which later extend to form a transparent white membrane.

Bouillon.—A slight cloudiness is produced, and a very scanty, whitish deposit is seen at the bottom of the tube. Does not develop well in acid bouillon.

Blood Serum.—A whitish layer is formed, which later becomes semi-transparent, and causes a slow liquefaction of the medium.

Potato.—At the end of forty-eight hours a layer is developed which has a velvety appearance in the centre, and a yellowish or brownish-white colour; by the end of forty-eight hours this layer is as large as a five-franc piece.

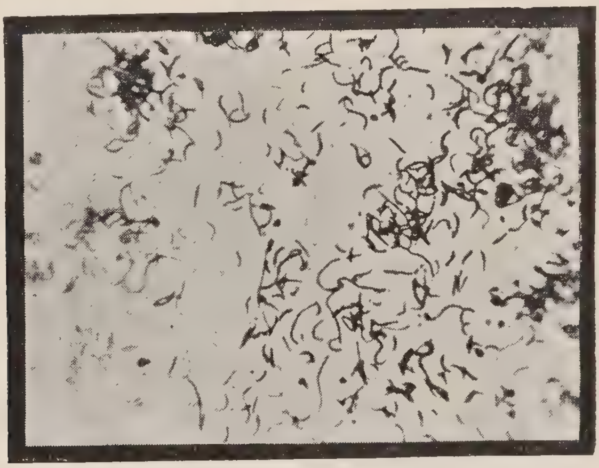

FIG. 72.—VIBRIO FINKLER-PRIOR.
From twenty-four-hours-old culture. × 1,000.

(42) VIBRIO FINKLER-PRIOR (V. PROTEUS).

Occasionally found in the mouth (Miller). Occurs in certain diarrhœa attacks.

Morphology.—Curved and bent rods 2·4 μ long, 0·4 to 0·6 μ broad; forms commas and spiral threads.

Staining Reactions.—Not by Gram, best with dilute carbol fuchsin or aniline gentian violet, and clearing in absolute alcohol.

Gelatin Plates, 22° C.—Round, edge entire, yellowish, granular. Microscopically, $\frac{2}{3}$ obj., yellow, centre darker, edge coarsely granular. Liquefaction in twenty-four hours. Occasionally the colonies have hair-like projections (ciliate).

Gelatin Stab, 22° C.—Saccate (sleeve shaped) liquefaction progressing very rapidly; the fluid remains turbid and a well marked pellicle is formed.

Agar Plate, 37·5° C.—Colonies similar to gelatin.

Agar Streak, 37·5 C.—Moist, yellowish rather; slimy and spreading.

Blood Serum, 37·5° C.—Moist, regular, well marked groove of liquefaction.

Potato, 22° C.—Well marked yellow, slimy, shining layer.

Litmus Milk, 37·5° C.—Coagulation of casein, which is redissolved; slight acid reaction.

Broth, 37·5° C.—General turbidity and pellicle formed. Indol reaction slight or absent.

Glucose Formate Broth, 37·5° C.—Grows anäerobically, but no gas produced.

(43) MICROCOCCUS ROSEUS (Bumm).
(*Diplococcus roseus, Flügge.*)

Widely distributed organism, very common in air, frequently present in mouth.

Morphology.—Round, oval and irregular cocci (0·6 to 1·0 μ in diameter), often occurring in masses or in pairs.

Staining Reactions.—Stains well with the ordinary aniline dyes and by Gram's method.

Biological Characters.—An äerobic, chromogenic coccus; gelatin slowly liquefied. Not motile (? Micrococcus agilis of Cohn). Grows best at 22° on ordinary media, also at 37·5° C. Pigment only formed in presence of air.

Gelatin Plates, 22° C.—Irregular, round or crenated, raised, small, rose-red colonies on surface, the deep colonies not developing much. The colonies gradually sink into the gelatin. Under $\frac{2}{3}$ obj., round or lenticular, entire edge, finely granular and pale rose-red in colour.

Gelatin Stab, 22° C.—Fine thread-like growth along stab, gelatin very slowly liquefied. Surface lobed and irregular, rose-red.

Gelatin Shake, 22° C.—Growth of colonies only near surface, little in depths. No gas.

Agar Streak, 37·5° C.—Smooth, shining, regular edge in twenty-four hours. Condensation water clear, and later, with red precipitate. Colour best developed at 20° C.

Potato, 22° C.—Glistening, rose-red, and often with outer white zone, often raised and lobular; medium not coloured.

Litmus Milk, 37·5° C.—No change.

Broth, 37·5° C.—Slight turbidity, with rose-red precipitate, coherent.

On potato cultures of Micrococcus roseus the colonies are a much brighter red.

A number of other cocci producing a red pigment have been described, but they are all apparently related to the Micrococcus roseus. Micrococcus lactericeus (Freund, *Cent. für Bakt.*, Bd. xxi., 834) differs slightly, but is probably a variety. Bacillus roseus (*Trans. Odont. Soc.*, June, 1898) is probably the same; the bacilli were very small and much resembled cocci.

The Sarcina roseus is thought by Lehmann and Neumann to be a "form" of the Micrococcus roseus (for further particulars see Lehmann and Neumann, p. 192).

APPENDIX.

The Microscope.

For bacteriological work a good compound microscope is necessary and should be fitted with the following :—

Objectives.—$\frac{2}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$ oil immersion.

Substage condenser.—Abbé or other pattern.

Nose piece.

Coarse and fine adjustment.

Mechanical stage.

The microscope consists of several parts which will be considered separately.

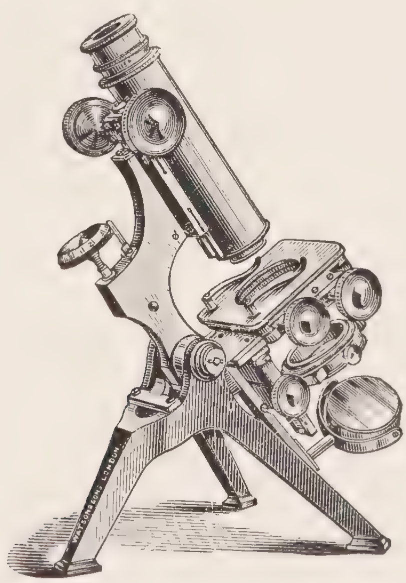

FIG. 73.—COMPOUND BACTERIOLOGICAL MICROSCOPE.

The Stand.—The pattern of stand is not of great importance, but the "tripod" is the most convenient for general work. It is most important that the stand be rigid and should allow of the body being tilted as far as the horizontal position in stable equilibrium.

The Stage.—Two species of stage are in use: (*a*) the plain, (*b*) the mechanical; and for bacteriological work the latter is

preferable, but not absolutely necessary. Those mechanical stages which are fixed by screws to the ordinary plain stage rarely work well for any length of time without developing a "kick," thereby throwing the object out of focus whenever the stage is adjusted. In selecting a stage care should be taken to observe that the movement in both directions is free from kick, and moreover sufficient to allow of plate cultivations, &c., being examined. This refers also to the plain stage.

The Substage Condenser.—Various forms of condenser are in use. The Abbé consists of a plano-convex and a concavo-convex lens and is the one generally in use. By means of the condenser the light is focussed upon the object, otherwise stained preparations cannot be brought sharply into focus when the $\frac{1}{12}$ obj. is in use. The condenser should be fitted with an iris diaphragm to regulate the light.

The Mirror.—Should have both plane and convex surfaces. The plane surface is to be used with the condenser.

Body Tube.—This tube carries the objectives and the eye-piece. The continental microscopes have a short body tube, the British a long tube, and the objectives are severally adapted. The body tube should be capable of extension, but it is essential to have a rack and pinion adjustment.

Focusing Adjustments.—The coarse adjustment is essential for all bacteriological work; by its action the objective is lowered till almost in focus and the focusing then completed with the fine adjustment. There are several forms of fine adjustments, and one should be chosen which does not carry more than half the weight of the body tube (see fig. 74).

The Objectives.—The $\frac{2}{3}$ obj. should give a perfectly flat field and sharp definition. The $\frac{1}{12}$ oil immersion lens should give good central definition with no blurring; the edges of the field must be free from colour refraction. The lens should also allow of the diaphragm to be opened to its full extent without causing any blurring of the image.

Great care is necessary in selecting lenses as it is impossible to make all lenses of uniform standard. Diatoms are not entirely satisfactory in testing a lens; blood films stained with eosin and bacteria stained with fuchsin give much better tests.

The oil should always be wiped off the $\frac{1}{12}$ after use with a clean piece of wash-leather kept for the purpose. If any dirt has been allowed to collect on the field lens it is best to clean it off with some immersion oil and the leather.

Only gross carelessness will account for canada balsam upon the $\frac{1}{12}$, and very great care must be exercised in removing it, otherwise the lens cement may be dissolved and the objective ruined.

Eye Pieces.—A low and high power eye-piece are convenient, the former magnifying about 5 diameters, the latter about 12.

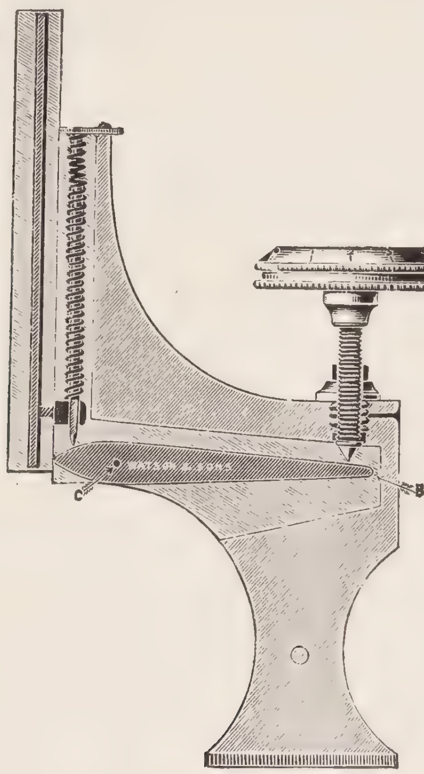

FIG. 74.—FINE ADJUSTMENT OF MICROSCOPE.

NOTES ON THE USE OF THE MICROSCOPE.

(1) *Unstained Specimens, Hanging Drop Slides, &c.*—Use the $\frac{1}{6}$ first always; it generally gives sufficient magnification for the purpose. Rack the lens down with the coarse adjustment until it almost, but not quite, touches the coverslip, then while looking through the microscope rack *upwards* until the object comes into view, then proceed with the fine adjustment. When using the condenser the flat side of the mirror should be employed, and with the $\frac{1}{6}$ obj. the condenser requires lowering.

(2) *Stained Specimens.*—Use the $\frac{1}{12}$ obj., taking care that no canada balsam is on the surface of the coverslip. Place a drop of immersion oil (cedar wood) in the middle of the coverslip, and with the coarse adjustment lower the lens till it touches the oil and *all but* touches the glass, then looking through the microscope

carefully rack *up* with the coarse adjustment until the object comes into view, finally focus with the fine adjustment. The diaphragm should be well open and the substage condenser raised to its limit.

Source of Light.—Daylight is the best, but—at any rate in London—is so uncertain that it is best to accustom oneself to a constant source of light. An argand burner or an incandescent mantle give the best results. It is an excellent plan to use a condenser in the form of a spherical flask, filled with water tinted with a neutral blue to correct the yellowness of the gas light; the water also removes the greater part of the heat rays. When not in use the microscope is advantageously kept covered with a glass bell-jar to exclude dust.

Hanging drop specimens should be made of all organisms examined. In examining the specimens under the microscope, rack down the condenser and use the $\frac{1}{6}$ obj. and close the iris diaphragm. All specimens of living bacteria require to be examined with the diaphragm nearly closed. With the stained specimens, on the other hand, rack the condenser close up to the slide and open the diaphragm when looking for the bacteria; with tissue preparations the diaphragm requires closing to bring out the tissue structure.

CLEANING APPARATUS.

Coverslips.—For smear preparations the coverslips must be entirely free from grease, &c., otherwise good specimens cannot be obtained.

The new slips are cleaned as follows:—

(1) Boil for thirty minutes in a strong solution of chromic acid.

(2) Wash with distilled water until no more yellow colour is seen in the washing water.

(3) Rinse in rectified spirit three times to remove the water.

(4) Wash in absolute alcohol twice.

(5) Transfer to a glass jar of absolute alcohol, using a pair of clean forceps which are kept for coverslips alone.

The coverslips must not be touched with the fingers when in alcohol.

Old coverslips, such as hanging drop preparations and slides, when finished with should be placed in 2 per cent. solution of lysol. The balsam becomes converted into soap and the slips are easily removed. They are then boiled in strong soap solution (Hudson's

Extract or Sapon) and then cleaned, as the new ones, in chromic acid, &c. The glass slips may be also boiled in soap solution and wiped dry with a clean cloth. The use of strong alkali spoils the glass.

Glass apparatus, beakers, Erlenmeyer flasks, &c., should be washed out with strong soap solution and a flask brush, rinsed and drained. Agar and gelatin if allowed to dry in the flasks is very difficult to remove.

CHARACTERS OF BACTERIAL CULTURES.

(From Chester's "Determinative Bacteriology.")

GELATIN STAB CULTURES.

A. Non-liquefying :—

Line of puncture.

Filiform.—Uniform growth with special characters (fig. 75, i.).

Nodose.—Consisting of closely aggregated colonies.

Beaded.—Consisting of loosely placed or disjointed colonies (fig. 75, ii.).

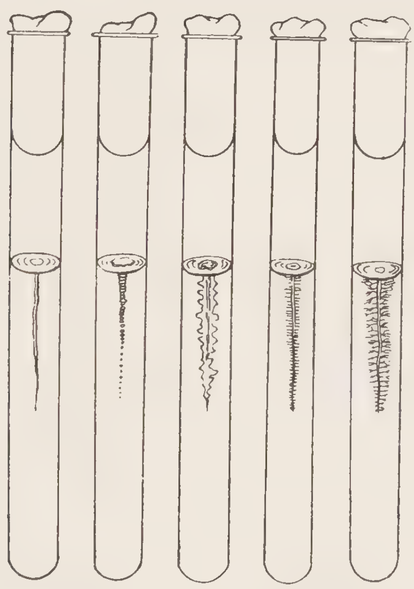

FIG. 75.—CHARACTER OF GELATIN STAB CULTURES.
i., Filiform; ii., beaded; iii., tuberculate-acinulate; iv., villous; v., arborescent. (Eyre, after Chester.)

Papillate.—Beset with papillate extensions.

Echinate.—Beset with acicular extensions (fig. 75, iii.).

Villous.—Beset with short, undivided, hair-like extensions (fig. 75, iv.).

Plumose.—Delicate feathery growth.

Arborescent.—Branched or tree-like, beset with branched hair-like extensions (fig. 75, v.).

B. Liquefying :—

Crateriform.—Saucer-shaped liquefaction of gelatin (fig. 76, i.).

Saccate.—Shaped like an elongated sac, tubular, cylindrical (fig. 76, ii.).

Infundibuliform.—Shaped like a funnel, conical (fig. 76, iii.).

Napiform.—Shaped like a turnip (fig. 76, iv.).

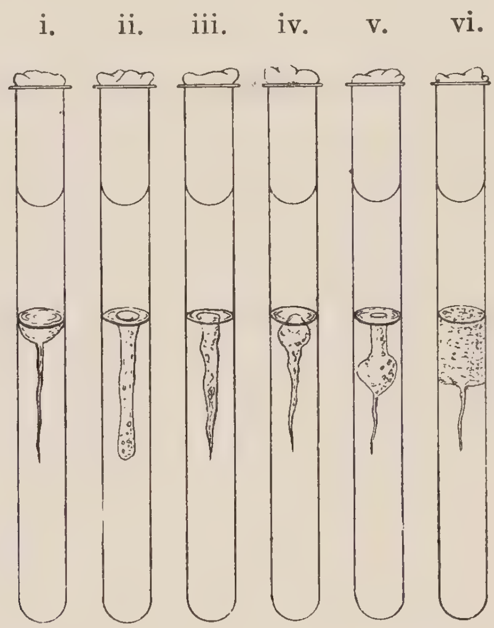

Fig. 76.—Characters of Liquefaction in Gelatin Stab Cultures.

i., Crateriform; ii., saccate; iii., infundibuliform; iv., napiform; v., fusiform; vi., stratiform. (Eyre, after Chester.)

Fusiform.—Outline of a parsnip, narrow either end, broadest below the surface (fig. 76, v.).

Stratiform.—Liquefaction extending to wall of tube and then downward horizontally (fig. 76, vi.).

Plate Cultures.

A. Form.

Punctiform.—Dimensions too slight for naked eye determination, minute, raised, semispherical.

Round.—Of more or less circular outline.

Irregular.

Elliptical.

Fusiform.—Spindle shaped, tapering either end.

Cochleate.—Spiral and twisted like a snail shell (fig. 77, i.).

Amœboid.—Very irregular streaming (Proteus) (fig. 77, ii.).

Mycelioid.—Filamentous, with the radiate character of a mould (fig. 77, iii.).

Filamentous.—An irregular mass of loosely interwoven filaments (fig. 78, i.).

Floccose.—A dense woolly structure.

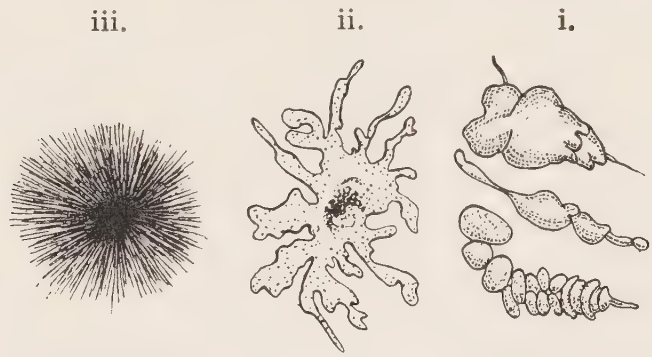

FIG. 77.—PLATE CULTURES: TYPES OF COLONIES (FORM).
i., Cochleate; ii., amœboid; iii., mycelioid. (Eyre, after Chester.)

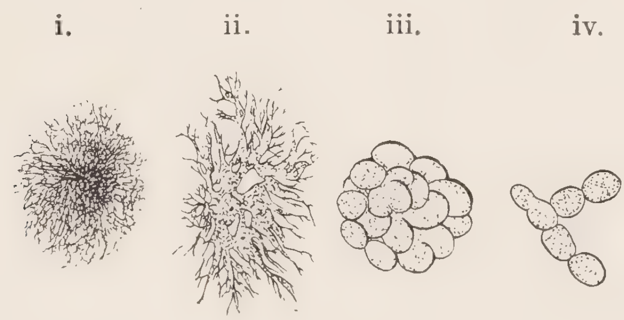

FIG. 78.—TYPES OF COLONIES: PLATE CULTURES (FORM).
i., Filamentous; ii., rhizoid; iii., conglomerate; iv., toruloid. (Eyre, after Chester.)

Rhizoid.—Of an irregular branched, root-like character (fig. 78, ii.).

Conglomerate.—An aggregation of colonies of similar size and form (fig. 78, iii.).

Toruloid.—An aggregation of colonies like a budding yeast plant (fig. 78, iv.).

Rosulate.—Shaped like a rosette.

B. Surface Elevation.

(1) General character as a whole.

Flat.—Thin leafy spreading over surface (fig. 79, i.).
Effused.—Spread over surface as a thin, veilly layer, more delicate than the preceding.
Raised.—Growth thick, with abrupt terraced edges (fig. 79, ii.).
Convex.—Surface the segment of a circle but very flat (fig. 79, iii.).
Pulvinate.—Surface the segment of a circle but decidedly convex (fig. 79, iv.).
Capitate.—Surface hemispherical (fig. 79, v.).

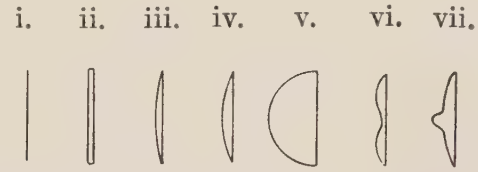

Fig. 79.—Characters of Surface Elevation of Plate Cultures, Non-Liquefying Stab Cultures.

i., Flat; ii., raised; iii., convex; iv., pulvinate; v., capitate; vi., umbilicate; vii., umbonate. (Eyre, after Chester.)

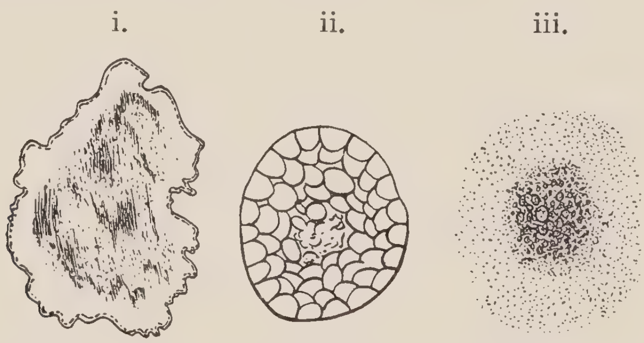

Fig. 80.—Structure of Colonies (Microscopic).

i., Clouded; ii., moruloid; iii., grumose in centre. (Eyre, after Chester.)

(2) Detailed characters of surface.

Smooth.—Surface even without any of the following distinctive characters.
Alveolate.—Marked by depressions separated by thin walls resembling a honeycomb (fig. 81, ii.).
Punctate.—Dotted with punctures like pin-pricks.
Bullate.—Like a blistered surface, rising in convex prominences, rather coarse.
Vesicular.—Covered with minute bubbles or vesicles due to gas, much finer than bullate.

Verrucose.—Wart-like, bearing wart-like prominences.

Squamous.—Scaly, covered with scales.

Echinate.—Beset with pointed prominences.

Papillate.—Beset with nipple-like projections.

Rugose.—Short irregular folds, due to shrinkage of surface growth.

Corrugated.—In long folds due to shrinkage.

Contoured.—An irregular but smoothly undulating surface, like the surface of a relief map.

Rimose.—Abounding in chinks, clefts or cracks.

C. *Internal Structure of Colony* (microscopic).

(1) Refraction weak.—Weak outline, and surface of relief not well defined.

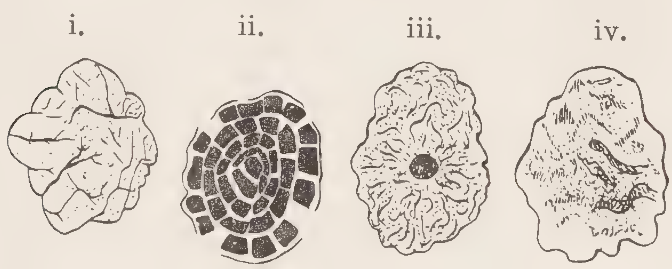

Fig. 81.—Structure of Colonies (Microscopic).
i., Reticulate; ii., alveolate; iii., gyrose; iv., marmorated. (Eyre, after Chester.)

(2) Refraction strong.—Outline and surface of relief well defined, dense not filamentous colonies.

General.

Amorphous.—Without definite structure as specified below.

Hyaline.—Clear and colourless.

Homogeneous.—Uniform structure throughout colony.

Homochromous.—Colony uniform throughout.

Finely Granular.

Coarsely Granular.

Grumose.—Coarser than preceding, particles in clustered grains (fig. 80, iii.).

Moruloid.—Segmented into more or less regular segments (fig. 80, ii.).

Clouded.—A pale ground with ill-defined patches of deeper colour (fig. 80, i.).

Reticulate.—In the form of network, like the veins of a leaf (fig. 81, i.).

Areolate.—Divided into rather irregular, angular spaces by more or less definite boundaries.

Marmorated.—Showing faint, irregular stripes, or traversed by vein-like markings as in marble (fig. 81, iv.).

Gyrose.—Marked by lines like the rivers of a map (fig. 81, iii.).

Rimose.—Showing chinks, cracks, or clefts.

Filamentous colonies.

Filamentous.—As already defined.

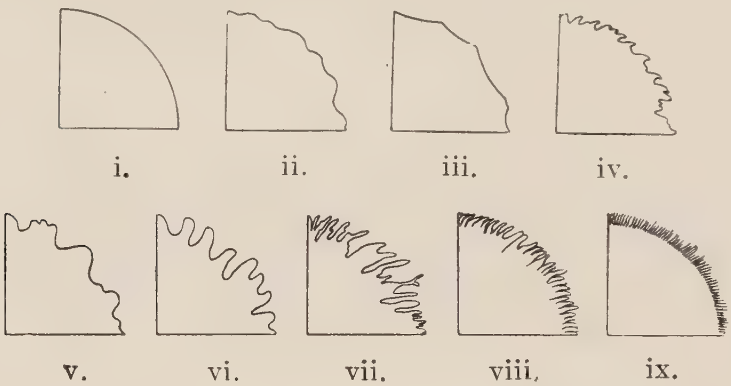

FIG. 82.—CHARACTERS OF EDGES OF COLONIES.

i., Entire; ii., undulate; iii., repand; iv., erose; v., lobate-lobulate; vi. auriculate; vii., lacerate; viii., fimbriate; ix., ciliate. (Eyre, after Chester.)

Floccose.—Filaments closely packed.

Curled.—Filaments in parallel strands like locks of hair or ringlets.

D. Edges of Colonies.

Entire.—Without toothing or division (fig. 82, i.).

Undulate.—Wavy (fig. 82, ii.).

Repand.—Like the border of an open umbrella (fig. 82, iii.).

Erose.—As if gnawed, irregularly toothed (fig. 82, iv.).

Lobate.—Blunt, rounded projection of edge (fig. 82, v.).

Lobulate.—Minutely lobate.

Auriculate.—With ear-like lobes (fig. 82, vi.).

Lacerate.—Irregularly cleft as if torn (fig. 82, vii.).

Fimbriate.—Fringed (fig. 82, viii.).

Ciliate.—Hair-like radial extensions (fig. 82, ix.).

Tufted.

Filamentous.
Curled.

Optical Characters :—

Transparent.—Transmitting light.
Vitreous.—Transparent and colourless.
Oleaginous.—Transparent and yellow, olive to linseed-oil coloured.
Resinous.—Transparent and brown varnish or resin coloured.
Translucent.—Faintly transparent.
Porcelainous.—Translucent and white.
Opalescent.—Translucent, greyish white by reflected light, smoky brown by transmitted light.
Nacreous.—Translucent, greyish white with pearly lustre.
Sebaceous.—Translucent, yellowish to greyish white.
Butyrous.—Translucent and yellow.
Ceraceous.—Opaque and wax coloured.
Cretaceous.—Opaque and chalky white.
Opaque.
Dull.—Without lustre.
Glistening.—Shining.
Fluorescent.
Iridescent.

CHEMICAL REACTIONS.

Indol.—Inoculate glucose free broth tubes with organism to be tested, incubate at 37·5° and 22° C. for ten days.

Test: Add ten drops of pure (nitrite free) concentrated sulphuric acid to each broth tube and 1 cc. of a 0·03 per cent. solution of sodium nitrite.

A pink colouration in ten minutes at room temperature indicates indol.

Phenol.—Place 50 cc. of the broth culture to be tested in a flask connected with a condenser, add 5 cc. pure concentrated hydrochloric acid. The distillate is collected and divided into three portions :—

(*a*) Add a few drops of Millon's reagent and boil; a red colour indicates phenol.

(*b*) Add a few drops of bromine water; turbidity if phenol is present.

(*c*) Add a few drops of dilute ferric chloride; a violet colour indicates phenol.

Reduction of Nitrates to Nitrites.

Inoculate medium composed of :—

Peptone	10 gm.
Sod. nitrate	0·02 gm.
Water	1,000 cc.

Test the water first and also use blank control tubes.

To the cultivation add a mixture of equal parts of the following solutions.—

I.—Naphthylamine	1·0 gm.
Water	100 cc.
II.—Sulphanilic acid	1·5 gm.
Dilute acetic acid	150 cc.

A pink coloration denotes reduction of nitrates to nitrites; the control tube often shows a pink colour, but if any change has occurred in the culture tube the colour is distinctly deeper.

Ammonia.—To 100 cc. of culture add 2 gm. of calcined magnesia, and distil. The distillate gives a yellow colour with Nessler's reagent, the tint of which is proportionate to the amount, and may be estimated in the usual way.

A control sample of uninoculated broth should also be distilled Nesslerised, and the tints compared.

Sulphuretted Hydrogen.—May be tested for by using broth or gelatin containing iron saccharate or tartrate; or a piece of lead acetate paper may be suspended in the culture tube.

Diastatic Ferment.—Inoculate sugar free broth, and after several days' incubation mix the culture with thin starch paste containing 2 per cent. thymol. Place the mixture in the incubator for eight to ten hours, filter and test filtrate with Fehling's solution.

For separation of acids see Chester's "Determinative Bacteriology," p. 39.

CHART FOR STUDY OF BACTERIA.

Designation	General Description	Source
MORPHOLOGY.		
Flagella		
Involution Forms		
Spores		
Staining Reactions		
BIOLOGY.		
Motility	Spore Germination	Agglutination
Anaerobiosis	Gas Production	Acid Production
Chromogensis	Aromatics	Enzyme Production
Optimum Temperature	Thermal Death Point	Optimum Reaction
CULTURAL REACTIONS.		
SOLID MEDIA.		
Agar Streak		
,, Stab		
Gelatin Streak		
,, Stab		
,, Plates		
,, Shake		
Potato		
Blood Serum		
LIQUID MEDIA.		
Broth		
Peptone Water		
Litmus Milk		*See also page* 69.
SPECIAL MEDIA.		

NOTES, PATHOGENESIS, ETC.

INDEX.

BENNETT.—*WORKS by Sir WILLIAM H. BENNETT, K.C.V.O., F.R.C.S., Surgeon to St. George's Hospital; Member of the Board of Examiners, Royal College of Surgeons of England.*

CLINICAL LECTURES ON VARICOSE VEINS OF THE LOWER EXTREMITIES. With 3 Plates. 8vo, 6*s*.

ON VARICOCELE: A PRACTICAL TREATISE. With 4 Tables and a Diagram. 8vo, 5*s*.

CLINICAL LECTURES ON ABDOMINAL HERNIA: chiefly in relation to Treatment, including the Radical Cure. With 12 Diagrams in the Text. 8vo, 8*s*. 6*d*.

ON VARIX, ITS CAUSES AND TREATMENT, WITH ESPECIAL REFERENCE TO THROMBOSIS: an Address delivered at the Inaugural Meeting of the Nottingham Medico-Chirurgical Society, Session 1898-9. 8vo, 3*s*. 6*d*.

ON THE USE OF MASSAGE AND EARLY PASSIVE MOVEMENTS IN RECENT FRACTURES AND OTHER COMMON SURGICAL INJURIES: THE TREATMENT OF INTERNAL DERANGEMENTS OF THE KNEE-JOINT: THE MANAGEMENT OF STIFF JOINTS: Three Clinical Lectures delivered at St. George's Hospital. With 17 Illustrations. 8vo, 6*s*.

THE PRESENT POSITION OF THE TREATMENT OF SIMPLE FRACTURES OF THE LIMBS: an Address delivered in opening a Discussion at the Meeting of the British Medical Association held at Ipswich, August, 1900. To which is appended a Summary of the Opinions and Practice of about 300 Surgeons. 8vo, 2*s*. 6*d*.

BENTLEY. A TEXT-BOOK OF ORGANIC MATERIA MEDICA. Comprising a Description of the Vegetable and Animal Drugs of the British Pharmacopœia, with some others in common use. Arranged Systematically, and Especially Designed for Students. By ROBERT BENTLEY, M.R.C.S. Eng., F.L.S. With 62 Illustrations on Wood. Crown 8vo, 7*s*. 6*d*.

BRODIE. THE ESSENTIALS OF EXPERIMENTAL PHYSIOLOGY. For the use of Students. By T. G. BRODIE, M.D., Lecturer on Physiology, St. Thomas's Hospital Medical School. With 2 Plates and 177 Illustrations in the Text. Crown 8vo, 6*s*. 6*d*.

CABOT. A GUIDE TO THE CLINICAL EXAMINATION OF THE BLOOD FOR DIAGNOSTIC PURPOSES. By RICHARD C. CABOT, M.D., Physician to Out-Patients, Massachusetts General Hospital. With 3 Coloured Plates and 28 Illustrations in the Text. 8vo, 16*s*.

CELLI. MALARIA ACCORDING TO THE NEW RESEARCHES. By Professor ANGELO CELLI, Director of the Institute of Hygiene, University of Rome. Translated from the Second Italian Edition by JOHN JOSEPH EYRE, M.R.C.P., L.R.C.S. Irel., D.P.H. Cambridge. With an Introduction by Dr. PATRICK MANSON, Medical Adviser to the Colonial Office. With 3 Maps and 40 Illustrations. 8vo, 10*s*. 6*d*.

CHEYNE AND BURGHARD. A MANUAL OF SURGICAL TREATMENT. By W. WATSON CHEYNE, C.B., M.B., F.R.C.S., F.R.S., Professor of Surgery in King's College, London; Surgeon to King's College Hospital, and the Children's Hospital, Paddington Green, etc.; and F. F. BURGHARD, M.D. and M.S. Lond., F.R.C.S., Teacher of Practical Surgery in King's College, London; Surgeon to King's College Hospital, and the Children's Hospital, Paddington Green, etc.

PROPOSED DIVISION OF WORK.

PART I. The treatment of General Surgical Diseases, including inflammation, suppuration, ulceration, gangrene, wounds and their complications, infective diseases and tumours; the administration of anæsthetics. With 66 Illustrations. Royal 8vo, 10*s*. 6*d*. [*Ready*.

PART II. The treatment of the Surgical Affections of the Tissues, including the skin and subcutaneous tissues, the nails, the lymphatic vessels and glands, the fasciæ, bursæ, muscles, tendons and tendon-sheaths, nerves, arteries and veins; deformities. With 141 Illustrations. Royal 8vo, 14*s*. [*Ready*.

PART III. The treatment of the Surgical Affections of the Bones. Amputations. With 100 Illustrations. Royal 8vo, 12*s*.

PART IV. The treatment of the Surgical Affections of the Joints (including excisions) and the spine. With 138 Illustrations. Royal 8vo, 14*s*.

PART V. The treatment of the Surgica Affections of the head, face, jawsl lips, larynx and trachea; and the Intrinsic Diseases of the nose, ear and larynx, by H. LAMBERT LACK, M.D. (Lond.), F.R.C.S., Surgeon to the Hospital for Diseases of the Throat, Golden Square, and to the Throat and Ear Department, the Children's Hospital, Paddington Green. With 145 Illustrations. Royal 8vo, 18*s*.

PART VI.—Section 1. The Surgical Affections of the tongue and floor of the mouth, the pharynx, neck, œsophagus, stomach and intestines. With 124 Illustrations. Royal 8vo, 18*s*.

Section 2. The Surgical Affections of the rectum, liver, spleen, pancreas, throat, breast and genito-urinary organs. With Illustrations. Royal 8vo. [*In preparation*.

CLARKE. POST-MORTEM EXAMINATIONS IN MEDICO-LEGAL AND ORDINARY CASES. With Special Chapters on the Legal Aspects of Post-Mortems, and on Certificates of Death. By J. JACKSON CLARKE, M.B. Lond., F.R.C.S., Assistant-Surgeon at the North-West London and City Orthopædic Hospitals, Pathologist and Curator of the Museum at St. Mary's Hospital. Fcap. 8vo, 2*s*. 6*d*.

COATS. A MANUAL OF PATHOLOGY. By JOSEPH COATS, M.D., late Professor of Pathology in the University of Glasgow. Fourth Edition. Revised throughout and Edited by LEWIS R. SUTHERLAND, M.D., Professor of Pathology, University of St. Andrews. With 490 Illustrations. 8vo, 31*s*. 6*d*.

*** A considerable portion of the work of revisal necessary in the preparation of a Fourth Edition of the above text-book had been already accomplished by the Author, Professor Joseph Coats, in the four months immediately preceding his death. It is in response to his expressed wish that the editorial duties have been assumed by Dr. Lewis R. Sutherland.

COOKE.—*WORKS by THOMAS COOKE, F.R.C.S. Eng., B.A., B.Sc., M.D. Paris, late Senior Assistant Surgeon to the Westminster Hospital.*

TABLETS OF ANATOMY. Being a Synopsis of demonstrations given in the Westminster Hospital Medical School. Eleventh Edition in three Parts, thoroughly brought up to date, and with over 700 Illustrations from all the best sources, British and Foreign. Post 4to. Part I. The Bones, 7*s.* 6*d.* net; Part II. Limbs, Abdomen, Pelvis, 10*s.* 6*d.* net; Part III. Head and Neck, Thorax, Brain, 10*s.* 6*d.* net.

APHORISMS IN APPLIED ANATOMY AND OPERATIVE SURGERY. Including 100 Typical *vivâ voce* Questions on Surface Marking, etc. Crown 8vo, 3*s.* 6*d.*

DISSECTION GUIDES. Aiming at Extending and Facilitating such Practical Work in Anatomy as will be specially useful in connection with an ordinary Hospital Curriculum. 8vo, 10*s.* 6*d.*

CURTIS. THE ESSENTIALS OF PRACTICAL BACTERIOLOGY: an Elementary Laboratory Book for Students and Practitioners. By H. J. CURTIS, B.S. and M.D. Lond., F.R.C.S., Assistant Surgeon, Royal Hospital for Children and Women, Waterloo Road; late Surgical Registrar, University College Hospital; formerly Assistant to the Professor of Pathology, University College, London. With 133 Illustrations. 8vo, 9*s.*

DAKIN. A HANDBOOK OF MIDWIFERY. By WILLIAM RADFORD DAKIN, M.D., F.R.C.P., Obstetric Physician and Lecturer o Midwifery at St. George's Hospital, Examiner in Midwifery and Diseases of Women on the Conjoint Board of the Royal Colleges of Physicians and Surgeons in England, etc. With 394 Illustrations. Large crown 8vo, 18*s.*

DICKINSON.—*WORKS by W. HOWSHIP DICKINSON, M.D. Cantab., F.R.C.P., Consulting Physician to St. George's Hospital; Consultin Physician to the Hospital for Sick Children, etc.*

ON RENAL AND URINARY AFFECTIONS. Complete in Three Parts, 8vo, with 12 Plates, and 122 Woodcuts. £3 4*s.* 6*d.*

THE TONGUE AS AN INDICATION OF DISEASE; being the Lumleian Lectures delivered at the Royal College of Physicians in March, 1888. 8vo, 7*s.* 6*d.*

OCCASIONAL PAPERS ON MEDICAL SUBJECTS, 1855-1896. 8vo, 12*s.*

MEDICINE OLD AND NEW: an Introductory Address delivered on the occasion of the Opening of the Winter Session, 1899-1900, at St. George's Hospital Medical School, on 2nd October, 1899. Crown 8vo, 2*s.* 6*d.*

ERICHSEN. THE SCIENCE AND ART OF SURGERY: A TREATISE ON SURGICAL INJURIES, DISEASES AND OPERATIONS. By Sir JOHN ERIC ERICHSEN, Bart., F.R.S., LL.D. Edin., Hon. M.Ch. and F.R.C.S. Ireland. Tenth Edition. Revised by the late MARCUS BECK, M.S. and M.B. Lond., F.R.C.S., Surgeon to University College Hospital, and Professor of Surgery in University College, London; and by RAYMOND JOHNSON, M.B. and B.S. Lond., F.R.C.S., Assistant Surgeon to University College Hospital, etc. Illustrated by nearly 1,000 Engravings on Wood. 2 vols. Royal 8vo, 48*s.*

FOWLER AND GODLEE. THE DISEASES OF THE LUNGS. By JAMES KINGSTON FOWLER, M.A., M.D., F.R.C.P., Physician to the Middlesex Hospital and to the Hospital for Consumption and Diseases of the Chest, Brompton; late Examiner in Medicine at the University of Cambridge, and on the Conjoint Examining Board in England; and RICKMAN JOHN GODLEE, M.S., F.R.C.S., Honorary Surgeon-in-Ordinary to His Majesty, Fellow and Professor of Clinical Surgery, University College, London; Surgeon to University College Hospital and to the Hospital for Consumption and Diseases of the Chest, Brompton; Surgeon-in-Ordinary to His Majesty's Household. With 160 Illustrations 8vo, 25*s*.

FRANKLAND. MICRO-ORGANISMS IN WATER, THEIR SIGNIFICANCE, IDENTIFICATION AND REMOVAL. Together with an Account of the Bacteriological Methods Involved in their Investigation. Specially Designed for the Use of those connected with the Sanitary Aspects of Water Supply. By Professor PERCY FRANKLAND, Ph.D., B.Sc. Lond., F.R.S., Fellow of the Chemical Society; and Mrs. PERCY FRANKLAND, Joint Author of "Studies on Some New Micro-Organisms Obtained from Air". With 2 Plates and numerous Diagrams. 8vo, 16*s*. net.

GARROD.—*WORKS by Sir ALFRED BARING GARROD, M.D., F.R.S., etc.; Consulting Physician to King's College Hospital; late Vice-President of the Royal College of Physicians.*

A TREATISE ON GOUT AND RHEUMATIC GOUT (RHEUMATOID ARTHRITIS). Third Edition, thoroughly Revised and Enlarged; with 6 Plates, comprising 21 figures (14 Coloured), and 27 Illustrations engraved on Wood. 8vo, 21*s*.

THE ESSENTIALS OF MATERIA MEDICA AND THERAPEUTICS. The Fourteenth Edition, Revised and Edited, under the Supervision of the Author, by NESTOR TIRARD, M.D. Lond., F.R.C.P., Professor of Materia Medica and Therapeutics in King's College, London, etc. Crown 8vo, 12*s*. 6*d*.

GOODSALL AND MILES. DISEASES OF THE ANUS AND RECTUM. By D. H. GOODSALL, F.R.C.S., Senior Surgeon Metropolitan Hospital, Senior Surgeon (late House Surgeon) St. Mark's Hospital; and W. ERNEST MILES, F.R.C.S., Assistant Surgeon to the Cancer Hospital, Surgeon (out-patients) to the Gordon Hospital, etc. (In Two Parts).

PART I.—Anatomy of the Ano-rectal Region—General Diagnosis—Abscess—Ano-rectal Fistula—Recto-urethral, Recto-vesical and Recto-vaginal Fistulæ—Sinus over the Sacro-coccygeal Articulation—Fissure—Hæmorrhoids (External and Internal). With 91 Illustrations. 8vo, 7*s*. 6*d*. net.

PART II.—(*Nearly ready*).

GRAY. ANATOMY, DESCRIPTIVE AND SURGICAL. By HENRY GRAY, F.R.S., late Lecturer on Anatomy at St. George's Hospital Medical School. The Fifteenth Edition Enlarged, edited by T. PICKERING PICK, F.R.C.S., Consulting Surgeon to St. George's Hospital, etc., and by ROBERT HOWDEN, M.A., M.B., C.M., Professor of Anatomy in the University of Durham, etc. With 772 Illustrations, a large proportion of which are Coloured, the Arteries being coloured red, the Veins blue, and the Nerves yellow. The attachments of the muscles to the bones, in the section on Osteology, are also shown in coloured outline. Royal 8vo, 32*s*. net.

HALLIBURTON.—*WORKS by W. D. HALLIBURTON, M.D., F.R.S., F.R.C.P., Professor of Physiology in King's College, London.*

A TEXT-BOOK OF CHEMICAL PHYSIOLOGY AND PATHOLOGY. With 104 Illustrations. 8vo, 28*s.*

THE ESSENTIALS OF CHEMICAL PHYSIOLOGY. For the Use of Students. Third Edition. With 77 Illustrations. 8vo, 5*s.*

The Liverpool University Press Publications.

THE THOMPSON YATES LABORATORIES REPORTS. Physiology; Pathology; Bacteriology; Tropical Medicine; Hygiene. Edited by RUBERT BOYCE and C. S. SHERRINGTON. With Plates and Illustrations in the Text. Demy 4to. Vol. I. 1898-9, 10*s.* 6*d.*; Vol. II. 1898-9, 25*s.*; Vol. III., Part I., 1900, 7*s.* 6*d.*; Vol. III., Part II., 1901, 12*s.* 6*d.*; Vol. IV., Part I., 1901, 20*s.*; Vol. IV., Part II., 1902, limp, 20*s.*; cloth, 21*s.*

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE MEMOIRS.

With Plates and Illustrations in the Text. Demy 4to.

I. MALARIAL FEVER: ITS CAUSE, PREVENTION AND TREATMENT. Containing full Details for the Use of Travellers, Sportsmen, Soldiers and Residents in Malarious Places. By RONALD ROSS, F.R.S., F.R.C.S. Ninth Edition, revised and enlarged. With Frontispiece. 8vo, 2*s.* 6*d.*

II. REPORT OF THE MALARIA EXPEDITION TO WEST AFRICA, AUGUST, 1899. By RONALD ROSS, D.P.H., M.R.C.S.; H. E. ANNETT, M.D., D.P.H., and E. E. AUSTEN. With Supplementary Reports by Major G. M. GILES, M.B., and R. FIELDING-OULD, M.B. 21*s.*

III. REPORT OF THE MALARIA EXPEDITION TO NIGERIA. By H. E. ANNETT, M.D.; J. EVERETT DUTTON, M.B., and J. H. ELLIOTT, M.D. Part I. Malarial Fever, etc. 10*s.* 6*d.*

IV. REPORT OF THE MALARIA EXPEDITION TO NIGERIA. By H. E. ANNETT, M.D.; J. EVERETT DUTTON, M.B., and J. H. ELLIOTT DUTTON, M.D. Part II. Filariasis. 15*s.*

V. PART 1. FIRST PROGRESS REPORT OF THE CAMPAIGN AGAINST MOSQUITOES IN SIERRA LEONE (1901). By Major R. ROSS, F.R.C.S., F.R.S. 8vo, 1*s.*

VII. REPORT OF THE YELLOW FEVER EXPEDITION TO PARÁ (1900). By H. E. DURHAM, M.B., F.R.C.S., and the late WALTER MYERS, M.B. 4to, 7*s.* 6*d.*

MISCELLANEOUS.

NOTES ON SANITARY CONDITIONS OBTAINING IN PARÁ. By the YELLOW FEVER EXPEDITION. 8vo, 1*s.*

LANG. THE METHODICAL EXAMINATION OF THE EYE. Being Part I. of a Guide to the Practice of Ophthalmology for Students and Practitioners. By WILLIAM LANG, F.R.C.S. Eng., Surgeon to the Royal London Ophthalmic Hospital, Moorfields, etc. With 15 Illustrations. Crown 8vo, 3*s*. 6*d*.

LIVEING. HANDBOOK ON DISEASES OF THE SKIN. With especial reference to Diagnosis and Treatment. By ROBERT LIVEING, M.A. and M.D. Cantab., F.R.C.P. Lond., etc., Physician to the Department for Diseases of the Skin at the Middlesex Hospital, etc. Fifth Edition, revised and enlarged. Fcap. 8vo, 5*s*.

LUFF. TEXT-BOOK OF FORENSIC MEDICINE AND TOXICOLOGY. By ARTHUR P. LUFF, M.D., B.Sc. Lond., Physician in Charge of Out-Patients and Lecturer on Medical Jurisprudence and Toxicology in St. Mary's Hospital; Examiner in Forensic Medicine in the University of London; External Examiner in Forensic Medicine in the Victoria University; Official Analyst to the Home Office. With 13 full-page Plates (1 in colours) and 33 Illustrations in the Text. 2 vols., Crown 8vo, 24*s*.

MOORE. ELEMENTARY PHYSIOLOGY. By BENJAMIN MOORE, M.A., Lecturer on Physiology at the Charing Cross Hospital Medical School. With 125 Illustrations. Crown 8vo, 3*s*. 6*d*.

NOTTER AND FIRTH. HYGIENE. By J. L. NOTTER, M.A., M.D., Professor of Hygiene in the Army Medical School, Netley; Colonel Royal Army Medical Corps; and R. H. FIRTH, F.R.C.S., late Assistant Professor of Hygiene in the Army Medical School, Netley; Major Royal Army Medical Corps. With 93 Illustrations. Crown 8vo, 3*s*. 6*d*.

PAGET. MEMOIRS AND LETTERS OF SIR JAMES PAGET, Bart., F.R.S., D.C.L., late Sergeant-Surgeon to Her late Majesty Queen Victoria. Edited by STEPHEN PAGET, F.R.C.S. With 6 Portraits (3 Photogravures) and 4 other Illustrations. 12*s*. 6*d*. net.

SELECTED ESSAYS AND ADDRESSES BY SIR JAMES PAGET. Edited by STEPHEN PAGET, F.R.C.S. 8vo, 12*s*. 6*d*. net.

Contents: Senile Scrofula (1867)—Cases that Bonesetters Cure (1867)—On Stammering with other Organs than those of Speech (1868)—What becomes of Medical Students (1869)—Sexual Hypochondriasis (1870)—On Dissection-wounds (1871)—Nervous Mimicry (1873)—On Disease of the Mammary Areola preceding Cancer of the Mammary Gland (1874)—On a Form of Chronic Inflammation of Bones (Osteitis Deformans) (1877)—Hunterian Oration (1877)—On some of the Sequels of Typhoid Fever (1879)—Anæsthetics: The History of a Discovery (1879)—Elementary Pathology (1880)—Theology and Science (1880)—Presidential Address at the Opening of the International Medical Congress (1881)—The Contrast of Temperance with Abstinence (1881)—Experiments on Animals (1881)—Some Rare and New Diseases (1882)—National Health and National Work (1884)—Periostitis following Strains (1891)—Spines Suspected of Deformity (1891)—Obscure Cases of Caries of the Spine (1891)—Errors in the Chronometry of Life (1891)—Use of the Will for Health (1891).

PICK. SURGERY: A TREATISE FOR STUDENTS AND PRACTITIONERS. By T. PICKERING PICK, Consulting Surgeon to St. George's Hospital; Senior Surgeon to the Victoria Hospital for Children; H.M. Inspector of Anatomy in England and Wales. With 441 Illustrations. Medium 8vo, 25*s.*

POOLE. COOKERY FOR THE DIABETIC. By W. H. and Mrs. POOLE. With Preface by Dr. PAVY. Fcap. 8vo, 2*s.* 6*d.*

POORE.—*WORKS by GEORGE VIVIAN POORE, M.D., F.R.C.P.*

THE EARTH IN RELATION TO THE PRESERVATION AND DESTRUCTION OF CONTAGIA: being the Milroy Lectures delivered at the Royal College of Physicians in 1899, together with other Papers on Sanitation. 13 Illustrations. Crown 8vo, 5*s.*

ESSAYS ON RURAL HYGIENE. Crown 8vo, 6*s.* 6*d.*

THE DWELLING HOUSE. With 36 Illustrations. Crown 8vo, 3*s.* 6*d.*

PROBYN-WILLIAMS. A PRACTICAL GUIDE TO THE ADMINISTRATION OF ANÆSTHETICS. By R. J. PROBYN-WILLIAMS, M.D., Anæsthetist and Instructor in Anæsthetics at the London Hospital; Lecturer in Anæsthetics at the London Hospital Medical College, etc. With 34 Illustrations. Crown 8vo., 4*s.* 6*d.* net.

QUAIN. QUAIN'S DICTIONARY OF MEDICINE. By Various Writers. Edited by H. MONTAGUE MURRAY, M.D., F.R.C.P., Joint Lecturer on Medicine, Charing Cross Medical School, and Physician to Out-Patients, Charing Cross Hospital; Senior Physician to the Victoria Hospital for Children, Chelsea, and to the Foundling Hospital. Assisted by JOHN HAROLD, M.B., B.CH., B.A.O., Physician to St. John's and St. Elizabeth's Hospital, and Demonstrator of Medicine at Charing Cross Medical School, and W. CECIL BOSANQUET, M.A., M.D., M.R.C.P., Physician to Out-Patients, Victoria Hospital for Children, Chelsea, and Pathologist to Charing Cross Hospital; late Fellow of New College, Oxford. Third and Cheaper Edition, largely Rewritten, and Revised throughout. With 21 Plates (14 in Colour) and numerous Illustrations in the Text. 8vo, 21*s.* net., buckram; 30*s.* net., half-morocco.

QUAIN. QUAIN'S (JONES) ELEMENTS OF ANATOMY. The Tenth Edition. Edited by EDWARD ALBERT SCHÄFER, F.R.S., Professor of Physiology in the University of Edinburgh; and GEORGE DANCER THANE, Professor of Anatomy in University College, London. In 3 vols.

*** The several parts of this work form COMPLETE TEXT-BOOKS OF THEIR RESPECTIVE SUBJECTS. They can be obtained separately as follows:—

VOL. I., PART I. EMBRYOLOGY. By E. A. SCHÄFER, F.R.S. With 200 Illustrations. Royal 8vo, 9*s.*

VOL. I., PART II. GENERAL ANATOMY OR HISTOLOGY. By E. A. SCHÄFER, F.R.S. With 291 Illustrations. Royal 8vo, 12*s.* 6*d.*

[*Continued.*

QUAIN. QUAIN'S (JONES) ELEMENTS OF ANATOMY—*cont.*

VOL. II., PART I. OSTEOLOGY — ARTHROLOGY. By G. D. THANE. With 224 Illustrations. Royal 8vo, 11*s.*

VOL. II., PART II. MYOLOGY — ANGEIOLOGY. By G. D. THANE. With 199 Illustrations. Royal 8vo, 16*s.*

VOL. III., PART I. THE SPINAL CORD AND BRAIN. By E. A. SCHÄFER, F.R.S. With 139 Illustrations. Royal 8vo, 12*s.* 6*d.*

VOL. III., PART II. THE NERVES. By G. D. THANE. With 102 Illustrations. Royal 8vo, 9*s.*

VOL. III., PART III. THE ORGANS OF THE SENSES. By E. A. SCHÄFER, F.R.S. With 178 Illustrations. Royal 8vo, 9*s.*

VOL. III., PART IV. SPLANCHNOLOGY. By E. A. SCHÄFER, F.R.S., and JOHNSON SYMINGTON, M.D. With 337 Illustrations. Royal, 8vo, 16*s.*

APPENDIX. SUPERFICIAL AND SURGICAL ANATOMY. By Professor G. D. THANE and Professor R. J. GODLEE, M.S. With 29 Illustrations. Royal 8vo, 6*s.* 6*d.*

SCHÄFER.—*WORKS by E. A. SCHÄFER, F.R.S., Professor of Physiology in the University of Edinburgh.*

THE ESSENTIALS OF HISTOLOGY: Descriptive and Practical. For the Use of Students. With 463 Illustrations. 8vo, 9*s.* net.

DIRECTIONS FOR CLASS WORK IN PRACTICAL PHYSIOLOGY: Elementary Physiology of Muscle and Nerve and of the Vascular and Nervous Systems. With 48 Diagrams. 8vo, 3*s.* net.

SCHENK. MANUAL OF BACTERIOLOGY. For Practitioners and Students. With especial reference to Practical Methods. By Dr. S. L. Schenk, Professor (Extraordinary) in the University of Vienna. Translated from the German, with an Appendix, by W. R. DAWSON, B.A., M.D. Univ. Dub.; late University Travelling Prizeman in Medicine. With 100 Illustrations, some of which are coloured. 8vo, 10*s.* net.

SMALE AND COLYER. DISEASES AND INJURIES OF THE TEETH, including Pathology and Treatment. By MORTON SMALE, M.R.C.S., L.S.A., L.D.S., Dental Surgeon to St. Mary's Hospital, Dean of the School, Dental Hospital of London, etc.; and J. F. COLYER, L.R.C.P., M.R.C.S., L.D.S., Dental Surgeon to Charing Cross Hospital and to the Dental Hospital of London. Second Edition Revised and Enlarged by J. F. COLYER. With 640 Illustrations. Large Crown 8vo, 21*s.*

SMITH (H. F.). THE HANDBOOK FOR MIDWIVES. By HENRY FLY SMITH, B.A., M.B. Oxon., M.R.C.S. Second Edition. With 41 Woodcuts. Crown 8vo, 5*s.*

STEVENSON. WOUNDS IN WAR: the Mechanism of their Production and their Treatment. By Surgeon-Colonel W. F. STEVENSON (Army Medical Staff), A.B., M.B., M.Ch. Dublin University; Professor of Military Surgery, Army Medical School, Netley. With 86 Illustrations. 8vo, 18*s.*

TAPPEINER. INTRODUCTION TO CHEMICAL METHODS OF CLINICAL DIAGNOSIS. By Dr. H. TAPPEINER, Professor of Pharmacology and Principal of the Pharmacological Institute of the University of Munich. Translated from the Sixth German Edition, with an Appendix on Micro-Biological Methods of Diagnosis, by EDMOND J. McWEENEY, M.A., M.D. Royal Univ. of Ireland, L.R.C.P.I., etc. With 22 Illustrations. Crown 8vo, 3*s.* 6*d.*

THORNTON. HUMAN PHYSIOLOGY. By JOHN THORNTON, M.A., Author of "Elementary Physiography," "Advanced Physiography," etc. With 267 Illustrations, some of which are Coloured. Crown 8vo, 6*s.*

WAKLEY. THE LIFE AND TIMES OF THOMAS WAKLEY. Founder and First Editor of the *Lancet*, Member of Parliament for Finsbury, and Coroner for West Middlesex. By S. SQUIRE SPRIGGE, M.B. Cantab. With 2 Portraits. 8vo, 6*s.*

WALLER.—*WORKS by AUGUSTUS D. WALLER, M.D., Lecturer on Physiology at St. Mary's Hospital Medical School, London; late External Examiner at the Victorian University.*

AN INTRODUCTION TO HUMAN PHYSIOLOGY. Third Edition, Revised. With 314 Illustrations. 8vo, 18*s.*

LECTURES ON PHYSIOLOGY.

FIRST SERIES.—*On Animal Electricity.* 8vo, 5*s.* net.

Veterinary Medicine, etc.

FITZWYGRAM. HORSES AND STABLES. By Lieutenant-General Sir F. FITZWYGRAM, Bart. With 56 pages of Illustrations. 8vo, 3*s.* net.

STEEL.—*WORKS by JOHN HENRY STEEL, F.R.C.V.S., F.Z.S., A.V.D., late Professor of Veterinary Science and Principal of Bombay Veterinary College.*

A TREATISE ON THE DISEASES OF THE DOG; being a Manual of Canine Pathology. Especially adapted for the use of Veterinary Practitioners and Students. With 88 Illustrations. 8vo, 10*s.* 6*d.*

A TREATISE ON THE DISEASES OF THE OX; being a Manual of Bovine Pathology. Especially adapted for the use of Veterinary Practitioners and Students. With 2 Plates and 117 Woodcuts. 8vo, 15*s.*

A TREATISE ON THE DISEASES OF THE SHEEP; being a Manual of Ovine Pathology for the use of Veterinary Practitioners and Students. With Coloured Plate and 99 Woodcuts. 8vo, 12*s.*

YOUATT.—*WORKS by WILLIAM YOUATT.*

THE HORSE. Revised and Enlarged by W. WATSON, M.R.C.V.S. With 52 Wood Engravings. 8vo, 6*s.*

THE DOG. Revised and Enlarged. With 33 Wood Engravings. 8vo, 6*s.*

General Scientific Works.

BEDDARD. THE STRUCTURE AND CLASSIFICATION OF BIRDS. By FRANK E. BEDDARD, M.A., F.R.S., Prosector and Vice-Secretary of the Zoological Society of London. With 252 Illustrations. 8vo, 21*s*. net.

BENNETT AND MURRAY. A HANDBOOK OF CRYPTOGAMIC BOTANY. By A. W. BENNETT, M.A., B.Sc., F.L.S., and GEORGE R. MILNE MURRAY, F.L.S. With 378 Illustrations. 8vo, 16*s*.

CHAPMAN. THE FORAMINIFERA: AN INTRODUCTION TO THE STUDY OF PROTOZOA. By FREDERICK CHAPMAN, A.L.S., F.R.M.S., formerly Assistant in the Geological Laboratory of the Royal College of Science; Palæontologist to the National Museum, Melbourne. With 14 Plates and 42 Illustrations in the Text. 8vo, 9*s*. net.

CROOKES. SELECT METHODS IN CHEMICAL ANALYSIS (chiefly inorganic). By Sir W. CROOKES, F.R.S., V.P.C.S. Editor of "The Chemical News". Third Edition, re-written and enlarged. Illustrated with 67 Woodcuts. 8vo, 21*s*. net.

CURTIS. A TEXT-BOOK OF GENERAL BOTANY. By CARLTON C. CURTIS, A.M., Ph.D., Tutor in Botany in Columbia University. With 87 Illustrations. Royal 8vo, 12*s*. net.

DRUDE. THE THEORY OF OPTICS. By PAUL DRUDE, Professor of Physics at the University of Giessen. Translated from the German by C. RIBORG MANN and ROBERT A. MILLIKAN, Assistant Professors of Physics at the University of Chicago. With 110 Diagrams. 8vo, 15*s*. net.

GANOT. ELEMENTARY TREATISE ON PHYSICS; Experimental and Applied, for the use of Colleges and Schools. Translated and edited from GANOT'S *Éléments de Physique* (with the Author's sanction) by E. ATKINSON, Ph.D., F.C.S., formerly Professor of Experimental Science, Staff College, Sandhurst. Fifteenth Edition, revised and enlarged, with 9 Coloured Plates and 1,057 Woodcuts. Large crown 8vo, 15*s*.

NATURAL PHILOSOPHY FOR GENERAL READERS AND YOUNG PEOPLE; Translated and Edited from GANOT'S *Cours Élémentaire de Physique* (with the Author's sanction) by E. ATKINSON, Ph.D., F.C.S. Ninth Edition, carefully revised by A. W. REINOLD, M.A., F.R.S., Professor of Physics in the Royal Naval College, Greenwich; with 7 Plates, 632 Woodcuts, and an Appendix of Questions. Crown 8vo, 7*s*. 6*d*.

GOODEVE.—*WORKS by T. M. GOODEVE, M.A., Barrister-at-Law; formerly Professor of Mechanics at the Normal School of Science and the Royal School of Mines.*

PRINCIPLES OF MECHANICS. New Edition, re-written and enlarged. With 253 Woodcuts and numerous Examples. Crown 8vo, 6*s*.

THE ELEMENTS OF MECHANISM. New Edition, re-written and enlarged. With 357 Woodcuts. Crown 8vo, 6*s*.

A MANUAL OF MECHANICS: an Elementary Text-Book for Students of Applied Mechanics. With 138 Illustrations and Diagrams, and 141 Examples taken from the Science Department Examination Papers with Answers. Fcap. 8vo, 2*s*. 6*d*.

HELMHOLTZ.—*WORKS by HERMANN L. F. HELMHOLTZ, M.D., late Professor of Physics in the University of Berlin.*

ON THE SENSATIONS OF TONE AS A PHYSIOLOGICAL BASIS FOR THE THEORY OF MUSIC. Second English Edition; with numerous additional notes and a new Additional Appendix, bringing down information to 1885, and specially adapted to the use of Musical Students. By ALEXANDER J. ELLIS, B.A., F.R.S., F.S.A., etc., formerly Scholar of Trinity College, Cambridge. With 68 Figures engraved on Wood, and 42 Passages in Musical Notes. Royal 8vo, 28*s.*

POPULAR LECTURES ON SCIENTIFIC SUBJECTS. With 68 Woodcuts. 2 vols. Crown 8vo, 3*s.* 6*d.* each.

HERSCHEL. OUTLINES OF ASTRONOMY. By Sir JOHN F. W. HERSCHEL, Bart., K.H., etc., Member of the Institute of France. Twelth Edition, with 9 Plates, and numerous Diagrams. 8vo, 12*s.*

HOFF. THE ARRANGEMENT OF ATOMS IN SPACE. By J. H. VAN 'T HOFF. Second, Revised and Enlarged Edition. With a Preface by JOHANNES WISLICENUS, Professor of Chemistry at the University of Leipzig; and an Appendix, "Stereochemistry among Inorganic Substances," by ALFRED WERNER, Professor of Chemistry at the University of Zürich. Translated and Edited by ARNOLD EILOART. Crown 8vo, 6*s.* 6*d.*

HUDSON AND GOSSE. THE ROTIFERA OR "WHEEL ANIMALCULES". By C. T. HUDSON, LL.D., and P. H. GOSSE, F.R.S. With 30 Coloured and 4 Uncoloured Plates. In 6 Parts. 4to, price 10*s.* 6*d.* each; Supplement, 12*s.* 6*d.* Complete in Two Volumes, with Supplement, 4to, £4 4*s.*

*** The Plates in the Supplement contain figures of almost all the Foreign Species, as well as of the British Species, that have been discovered since the original publication of Vols. I. and II.

JOUBERT. ELEMENTARY TREATISE ON ELECTRICITY AND MAGNETISM. Founded on JOUBERT'S "*Traité Élémentaire d'Electricité*". By G. C. FOSTER, F.R.S., Quain Professor of Physics in University College, London; and E. ATKINSON, Ph.D., formerly Professor of Experimental Science in the Staff College. With Illustrations. Crown 8vo. [*New Edition in the Press.*

KOLBE. A SHORT TEXT-BOOK OF INORGANIC CHEMISTRY. By Dr. HERMANN KOLBE, late Professor of Chemistry in the University of Leipzig. Translated and Edited by T. S. HUMPIDGE, Ph.D., B.Sc. Lond., late Professor of Chemistry and Physics in the University College of Wales, Aberystwyth. New Edition. Revised by H. LLOYD-SNAPE, Ph.D., D.Sc. Lond., Professor of Chemistry in the University College of Wales, Aberystwyth. With a Coloured Table of Spectra and 66 Woodcuts. Crown 8vo, 8*s.* 6*d.*

LARDEN. ELECTRICITY FOR PUBLIC SCHOOLS AND COLLEGES. With numerous Questions and Examples with Answers, and 214 Illustrations and Diagrams. By W. LARDEN, M.A. Crown 8vo, 6*s.*

LINDLEY AND MOORE. THE TREASURY OF BOTANY, OR POPULAR DICTIONARY OF THE VEGETABLE KINGDOM : with which is incorporated a Glossary of Botanical Terms. Edited by J. LINDLEY, M.D., F.R.S., and T. MOORE, F.L.S. With 20 Steel Plates and numerous Woodcuts. 2 Parts. Fcap. 8vo, 12*s.*

LOWELL. MARS. By PERCIVAL LOWELL, Fellow American Academy, Member Royal Asiatic Society, Great Britain and Ireland, etc. With 24 Plates. 8vo, 12*s.* 6*d.*

*** The book is written in a style suitable for the general reader, and the most recent speculations as to the planet being inhabited, the possible canals, oases, etc., are discussed.

MACDOUGAL.—*WORKS by DANIEL TREMBLY MACDOUGAL, Ph.D., Director of the Laboratories of the New York Botanical Garden.*

PRACTICAL TEXT-BOOK OF PLANT PHYSIOLOGY. With 159 Illustrations. 8vo, 7*s.* 6*d.* net.

ELEMENTARY PLANT PHYSIOLOGY. With 108 Illustrations. Crown 8vo, 3*s.*

MARTIN. NAVIGATION AND NAUTICAL ASTRONOMY. Compiled by Staff-Commander W. R. MARTIN, R.N., Instructor in Surveying, Navigation and Compass Adjustment ; Lecturer on Meteorology at the Royal Naval College, Greenwich. Sanctioned for use in the Royal Navy by the Lords Commissioners of the Admiralty. Royal 8vo, 18*s.*

MENDELÉEFF. THE PRINCIPLES OF CHEMISTRY. By D. MENDELÉEFF. Translated from the Russian (Sixth Edition) by GEORGE KAMENSKY, A.R.S.M., of the Imperial Mint, St. Petersburg, and Edited by T. A. LAWSON, B.Sc., Ph.D., Fellow of the Institute of Chemistry. With 96 Diagrams and Illustrations. 2 vols. 8vo, 36*s.*

MEYER. OUTLINES OF THEORETICAL CHEMISTRY. By LOTHAR MEYER, Professor of Chemistry in the University of Tübingen. Translated by Professors P. PHILLIPS BEDSON, D.Sc., and W. CARLETON WILLIAMS, B.Sc. 8vo, 9*s.*

MEYER. THE KINETIC THEORY OF GASES. Elementary Treatise with Mathematical Appendices. By Dr. OSKAR EMIL MEYER, Professor of Physics at the University of Breslau. Second Revised Edition. Translated by ROBERT E. BAYNES, M.A., Student of Christ Church, Oxford, and Dr. Lee's Reader in Physics. 8vo, 15*s.* net.

MORGAN. ANIMAL BIOLOGY. An Elementary Text-Book. By C. LLOYD MORGAN, F.R.S., Principal of University College, Bristol. With numerous Illustrations. Crown 8vo, 8*s.* 6*d.*

OSTWALD. SOLUTIONS. By W. OSTWALD, Professor of Chemistry in the University of Leipzig. Being the Fourth Book, with some additions, of the Second Edition of Ostwald's " Lehrbuch der Allgemeinen Chemie ". Translated by M. M. PATTISON MUIR, Professor of Gonville and Caius College, Cambridge. 8vo, 10*s.* 6*d.*

PROCTOR.—*WORKS by RICHARD A. PROCTOR.*

LIGHT SCIENCE FOR LEISURE HOURS; Familiar Essays on Scientific Subjects, Natural Phenomena, etc. Crown 8vo, 3*s.* 6*d.*

THE ORBS AROUND US; a Series of Essays on the Moon and Planets, Meteors and Comets. With Chart and Diagrams. Crown 8vo, 3*s.* 6*d.*

OTHER WORLDS THAN OURS; The Plurality of Worlds Studied under the Light of Recent Scientific Researches. With 14 Illustrations. Crown 8vo, 3*s.* 6*d.*

THE MOON; her Motions, Aspects, Scenery and Physical Condition. With Plates, Charts, Woodcuts and Lunar Photographs. Crown 8vo, 3*s.* 6*d.*

LARGER STAR ATLAS for the Library, in 12 Circular Maps, with Introduction and 2 Index Pages. Folio, 15*s.*, or Maps only, 12*s.* 6*d.*

NEW STAR ATLAS for the Library, the School and the Observatory, in 12 Circular Maps (with 2 Index Plates). Crown 8vo, 5*s.*

OTHER SUNS THAN OURS: a Series of Essays on Suns—Old, Young and Dead. With other Science Gleanings, Two Essays on Whist, and Correspondence with Sir John Herschel. With 9 Star-Maps and Diagrams. Crown 8vo, 3*s.* 6*d.*

HALF-HOURS WITH THE TELESCOPE: a Popular Guide to the Use of the Telescope as a Means of Amusement and Instruction. With 7 Plates. Fcap. 8vo, 2*s.* 6*d.*

THE EXPANSE OF HEAVEN. Essays on the Wonders of the Firmament. Crown 8vo, 3*s.* 6*d.*

PLEASANT WAYS IN SCIENCE. Crown 8vo, 3*s.* 6*d.*

THE SOUTHERN SKIES: a Plain and Easy Guide to the Constellations of the Southern Hemisphere. Showing in 12 Maps the Position of the principal Star-Groups night after night throughout the year. With an Introduction and a separate Explanation of each Map. True for every Year. 4to, 5*s.*

MYTHS AND MARVELS OF ASTRONOMY. Crown 8vo, 3*s.* 6*d.*

HALF-HOURS WITH THE STARS: a Plain and Easy Guide to the Knowledge of the Constellations. Showing in 12 Maps the position of the principal Star-Groups night after night throughout the Year. With Introduction and a separate Explanation of each Map. True for every Year. 4to, 3*s.* net.

THE STARS IN THEIR SEASONS. An Easy Guide to a Knowledge of the Star Groups, in 12 large Maps. Imperial 8vo, 5*s.*

OUR PLACE AMONG INFINITIES: a Series of Essays contrasting our Little Abode in Space and Time with the Infinities around Us. Crown 8vo, 3*s.* 6*d.*

ROUGH WAYS MADE SMOOTH. Familiar Essays on Scientific Subjects. Crown 8vo, 3*s.* 6*d.*

NATURE STUDIES. By GRANT ALLEN, A. WILSON, T. FOSTER, E. CLODD and R. A. PROCTOR. Crown 8vo, 3*s.* 6*d.*

LEISURE READINGS. By E. CLODD, A. WILSON, T. FOSTER, A. C. RUNYARD and R. A. PROCTOR. Crown 8vo, 3*s.* 6*d.*

STRENGTH: How to get Strong and keep Strong, with Chapters on Rowing and Swimming, Fat, Age and the Waist. With 9 Illustrations. Crown 8vo, 2*s.*

REYNOLDS. EXPERIMENTAL CHEMISTRY for Junior Students. By J. EMERSON REYNOLDS, M.D., F.R.S., Professor of Chemistry, Univ. of Dublin. Fcap. 8vo, with numerous Woodcuts.

PART I.—*Introductory*, 1*s.* 6*d.* PART III.—*Metals and Allied Bodies*, 3*s.* 6*d.*

PART II.—*Non-Metals*, 2*s.* 6*d.* PART IV.—*Chemistry of Carbon Compounds*, 4*s.*

ROMANES.—*WORKS by GEORGE JOHN ROMANES, M.A., LL.D., F.R.S.*

DARWIN, AND AFTER DARWIN : an Exposition on the Darwinian Theory, and a Discussion on Post-Darwinian Questions. Part I. The Darwinian Theory. With Portrait of Darwin and 125 Illustrations. Crown 8vo, 10*s.* 6*d.* Part II. Post-Darwinian Questions : Heredity and Utility. With Portrait of the Author and 5 Illustrations. Crown 8vo, 10*s.* 6*d.* Part III. Post-Darwinian Questions : Isolation and Physiological Selection. Crown 8vo, 5*s.*

AN EXAMINATION OF WEISMANNISM. Crown 8vo, 6*s.*

ESSAYS. Edited by C. LLOYD MORGAN, F.R.S., Principal of University College, Bristol. Crown 8vo, 6*s.*

Contents : Primitive Natural History—The Darwinian Theory of Instinct—Man and Brute—Mind in Men and Animals—Origin of Human Faculty—Mental Differences between Men and Women—What is the Object of Life ?—Recreation—Hypnotism—Hydrophobia and the Muzzling Order.

SLINGO AND BROOKER. ELECTRICAL ENGINEERING FOR ELECTRIC-LIGHT ARTISANS AND STUDENTS. (Embracing those branches prescribed in the Syllabus issued by the City and Guilds Technical Institute.) By W. SLINGO and A. BROOKER. With 371 Illustrations. Crown 8vo, 12*s.*

SORAUER. A POPULAR TREATISE ON THE PHYSIOLOGY OF PLANTS. For the Use of Gardeners or for Students of Horticulture and of Agriculture. By Dr. PAUL SORAUER, Director of the Experimental Station at the Royal Pomological Institute in Proskau (Silesia). Translated by F. E. WEISS, B.Sc., F.L.S., Professor of Botany at the Owens College, Manchester. With 33 Illustrations. 8vo, 9*s.* net.

THORPE. A DICTIONARY OF APPLIED CHEMISTRY. By T. E. THORPE, C.B., B.Sc. Vict., Ph.D., F.R.S., Treas. C.S., Director of Government Laboratories, London. Assisted by Eminent Contributors. To be published in 3 vols. 8vo. Vols. I. and II., £2 2*s.* each ; Vol. III., £3 3*s.*

TILDEN. A SHORT HISTORY OF THE PROGRESS OF SCIENTIFIC CHEMISTRY IN OUR OWN TIMES. By WILLIAM A. TILDEN, D.Sc. Lond., D.Sc. Dub., F.R.S., Fellow of the University of London, Professor of Chemistry in the Royal College of Science, London. Crown 8vo, 5*s.* net.

TUBEUF. DISEASES OF PLANTS DUE TO CRYPTOGAMIC PARASITES. Translated from the German of Dr. CARL FREIHERR VON TUBEUF, of the University of Munich, by WILLIAM G. SMITH, B.Sc., Ph.D., Lecturer on Plant Physiology to the University of Edinburgh. With 330 Illustrations. Royal 8vo, 18*s.* net.

TYNDALL.—*WORKS by JOHN TYNDALL, F.R.S., etc.*

FRAGMENTS OF SCIENCE. 2 Vols. Crown 8vo, 16*s*.

NEW FRAGMENTS. Crown 8vo, 10*s*. 6*d*.

HEAT A MODE OF MOTION. Crown 8vo, 12*s*.

SOUND. With 204 Woodcuts. Crown 8vo, 10*s*. 6*d*.

RESEARCHES ON DIAMAGNETISM AND MAGNE-CRYSTALLIC ACTION, including the question of Diamagnetic Polarity. Crown 8vo, 12*s*.

ESSAYS ON THE FLOATING MATTER OF THE AIR in relation to Putrefaction and Infection. With 24 Woodcuts. Crown 8vo, 7*s*. 6*d*.

LECTURES ON LIGHT, delivered in America in 1872 and 1873. With 57 Diagrams. Crown 8vo, 5*s*.

LESSONS IN ELECTRICITY AT THE ROYAL INSTITUTION, 1875-76. With 58 Woodcuts. Crown 8vo, 2*s*. 6*d*.

NOTES OF A COURSE OF SEVEN LECTURES ON ELECTRICAL PHENOMENA AND THEORIES, delivered at the Royal Institution. Crown 8vo, 1*s*. 6*d*.

NOTES OF A COURSE OF NINE LECTURES ON LIGHT, delivered at the Royal Institution. Crown 8vo, 1*s*. 6*d*.

FARADAY AS A DISCOVERER. Crown 8vo, 3*s*. 6*d*.

THE GLACIERS OF THE ALPS: being a Narrative of Excursions and Ascents. An Account of the Origin and Phenomena of Glaciers and an Exposition of the Physical Principles to which they are related. With 61 Illustrations. Crown 8vo, 6*s*. 6*d*. net.

WATTS (H.). DICTIONARY OF CHEMISTRY. Revised and entirely Re-written by H. FORSTER MORLEY, M.A., D.Sc., Fellow of, and lately Assistant-Professor of Chemistry in, University College, London; and M. M. PATTISON MUIR, M.A., F.R.S.E., Fellow and Prælector in Chemistry of Gonville and Caius College, Cambridge. Assisted by Eminent Contributors. 4 vols. 8vo, £5 net.

WEBB. CELESTIAL OBJECTS FOR COMMON TELESCOPES. By the Rev. T. W. WEBB, M.A., F.R.A.S., late Vicar of Hardwick, Herefordshire. Fifth Edition, Revised and greatly Enlarged by the Rev. T. E. ESPIN, M.A., F.R.A.S. (Two Volumes.)

VOL. I. With Portrait and a Reminiscence of the Author, 2 Plates and numerous Illustrations. Crown 8vo, 6*s*.

VOL. II. With Illustrations and Map of Star Spectra. Crown 8vo, 6*s*. 6*d*.

WRIGHT. OPTICAL PROJECTION: A Treatise on the Use of the Lantern in Exhibition and Scientific Demonstration. By LEWIS WRIGHT, Author of "Light: a Course of Experimental Optics". With 232 Illustrations. Crown 8vo, 6*s*.

5,000. 6/02. THE ABERDEEN UNIVERSITY PRESS LIMITED.